Original Research Paper

TGF-beta1 is associated with the progression of intracranial deep white matter lesions: a pilot study with 5 years of magnetic resonance imaging follow-up

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Objectives: Elevated expression of transforming growth factor (TGF)-beta1 has been reported in hereditary cerebral small-vessel (HCSV) disease. The aim of this study was to clarify whether TGF-beta1 is a risk factor for intracranial deep white matter lesions (DWLs) and their progression in a general elderly population.

Methods: The subjects included 81 participants (Groups DWL, DWLP, and C) who had voluntarily undergone a health examination and brain magnetic resonance imaging (MRI) in 2003 and 2008 and 43 age-matched patients with previous symptomatic brain infarctions. Deep white matter lesions were graded from Grade 0 to 3 according to the Fazekas classification. Group DWL (23 subjects) was defined as DWLs with no progression in the grade level, and Group DWLP (progression of DWL) (12 subjects) was defined as DWLs with an increase in one or more grade number and an apparent worsening of Grade 3.
Forty-six age-matched control subjects with consistent normal brain MRI were included in Group C. The associations between DWLs and various vascular risk factors, including peripheral blood TGF-beta1 levels, were examined.

Results: In addition to the classical risk factors, the highest TGF-beta1 levels were found in Group DWLP. The TGF-beta1 levels were significantly higher in Group DWLP than in Group DWL, and DWLP was significantly correlated with elevated TGF-beta1 levels (odds ratio [OR]=1.72).

Conclusions: The present data suggest that TGF-beta1 may be important in the pathogenesis and progression of DWLs, and it is expected to be useful as a clinical indicator reflecting the presence of intracranial white matter lesions.

Keywords: Deep white matter lesions (DWLs), Progression, TGF-beta1

Introduction

Recent pathological studies have shown that cerebral deep white matter lesions (DWLs) on magnetic resonance imaging (MRI) are related to intracranial small-vessel disease.[1] With the dissemination of MRI, DWLs have come to be frequently detected in healthy, elderly subjects. Yao *et al.*[2] performed a brain MRI study in healthy community residents and reported asymptomatic DWLs in 34%, while a clinical epidemiological study by Gouw *et al.*[3] showed DWL progression (DWLP) in 21% of community residents at a 3-year follow-up with repeated brain MRI.

In 2009, Hara *et al.*[4] reported that hereditary cerebral small-vessel (HCSV) disease with DWLP was strongly linked to an abnormal transforming growth factor (TGF)-beta1 signaling pathway due to a mutation in the HtrA serine peptidase 1 (*HTRA1*) gene, and the pathology of HCSV was noted to be similar to that of sporadic cerebral small-vessel disease (SVD). This implies that *HTRA1* is a candidate gene for a type of HCSV called CARASIL (cerebral autosomal recessive arteriopathy

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with subcortical infarcts and leukoencephalopathy), and it also implies an unexpected common mechanism between HCSV with DWLs and SVD. This suggests that we should focus more carefully on the genetic factors underlying SVD.

In addition, twin and family studies have suggested that SVD characterized by DWLs on MRI examination is one of the most heritable cerebrovascular phenotypes among twins or family members, apart from classical risk factors such as hypertension.[5] Interestingly, *HTRA1* has also been reported to be a serine protease that directly represses signaling by TGF-beta family members,[6] and the considerable effects of TGF-beta1 on blood vessel formation, e.g. acting as a pro-angiogenic factor in small arteries of SVD, have also been reported.[7–9] Taking these reports together, we hypothesized that TGF-beta1 signaling is somehow associated with the pathogenesis of SVD. Moreover, TGF-beta1 expression is of importance in the pathogenesis of atherosclerosis.[10,11]

The aim of this study was to clarify retrospectively whether TGF-beta1 levels and related atherosclerotic vascular components are risk factors for the progression of intracranial DWLs in a general elderly population.

Materials and Methods

This study was approved in advance by the ethics committee of the university, and all subjects gave their written, informed consent.

In this prospective case–control study, 486 elderly individuals in Kyoto Prefecture participated voluntarily in a hospital-based health check-up following a random mailing recruitment approach, and 291 participants voluntarily agreed to undergo repeated brain MRI examinations in 2003 and 2008. Following the MRI evaluations in 2003, 45 subjects were found to have DWLs. The general background of this project has been described previously.[12] In this study, we recruited all of the 45 subjects who agreed to undergo repeated brain MRI in 2003 and 2008 through a health consultation program at the health check-up center in 2003. During the 5-year follow-up period, six subjects refused to continue attending and four dropped out spontaneously. Thus, 35 subjects with DWLs were finally evaluated in this study and were classified as Groups DWL (23 subjects) and DWLP (12 subjects) (Table 1). These two groups were categorized separately in accordance with the MRI findings described later in the 'Evaluation of Brain MRI' section. Simultaneously, 46 unimpaired, age-matched control subjects (Group C) with normal brain MRI examinations in 2003 and 2008 were also enrolled through the same random mailing recruitment approach (Table 1).

In addition, to check for and eliminate the possible latent effects of TGF-beta1 elevation on intracranial atherosclerosis induced by a past history of infarction, 43 age-matched outpatients with previous symptomatic brain infarctions were also recruited separately from our clinic to participate in this study. These patients were assigned to two groups according to the criteria of the TOAST classification, which is commonly used as an etiological classification for ischemic stroke:[13] Group L (27 subjects) comprised those who exhibited lacunar infarctions; and Group A

Table 1 Clinical characteristics and transforming growth factor (TGF)-beta1 levels by group and logistic regression of variables in the DWL and DWLP groups

	C	L	A	DWL OR (95% CI)	DWLP OR (95% CI)
Numbers	46	27	16	23	12
Age (mean ± SD)	67.4 ± 3.5	71.2 ± 5.8	69.1 ± 7.1	68.6 ± 6.6	69.1 ± 4.0
Males/females	28/18	18/9	12/4	13/10	7/5
HT (%)	7 (15.2)	15 (55.6)**	8 (50.0)**	12 (52.2)**	7 (58.3)**
	ref			2.13 (1.76–4.95)*	2.37 (1.43–5.19)*
DM (%)	5 (10.9)	7 (25.9)	5 (31.3)	8 (35.0)*	7 (58.3)**
	ref			1.71 (1.02–3.13)*	1.96 (1.28–4.07)*
HL (%)	9 (19.6)	13 (48.1)**	8 (50.0)**	10 (43.5)**	4 (33.3)*
	ref			1.91 (1.28–3.50)*	1.84 (1.25–2.97)*
Smoking (%)	7 (15.2)	3 (11.1)	1 (6.3)	5 (21.7)	3 (25.0)
	ref			0.87 (0.64–2.54)	0.94 (0.62–2.93)
IHD (%)	1 (2.2)	6 (22.2) *	5 (31.3)	1 (4.3)	1 (8.3)
	ref			0.76 (0.47–2.18)	0.91 (0.58–3.15)
PVH (%)	5 (10.9)	6 (22.2)	6 (37.5) *	7 (30.4)*	4 (33.3)*
	ref			1.21 (1.03–3.02)*	1.52 (1.22–2.90)*
TGF-beta1 (>35 ng/ml) (%)	2 (4.3)	2 (7.4)	1 (6.3)	3 (13.0)	8 (66.7)**
	ref			1.03 (0.51–1.89)	1.72 (1.29–4.52)*

Odds ratio (OR) indicates crude odds ratio versus Group C on logistic regression analysis.
Group C, control; Group DWL, group with deep white matter lesions; Group DWLP, progression of DWLs; Group L, lacunar infarction; Group A, atherothrombotic infarction; HT, hypertension; DM, diabetes mellitus; HL, hyperlipidemia; IHD, ischemic heart disease; PVH, periventricular hyperintensity; CI, confidence interval; ref, reference.
*$P<0.05$; **$P<0.01$ versus Group C. *$P<0.05$ versus Group C.

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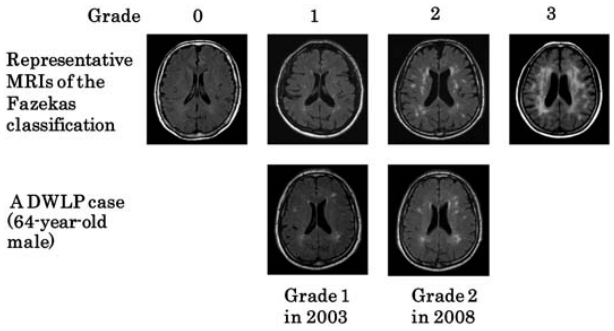

Figure 1 (Upper): Representative deep white matter lesion (DWL) cases according to the magnetic resonance imaging (MRI)-based Fazekas classification. The definition of DWLs using the MRI-based Fazekas classification applied in this study is shown in this figure. Deep white matter lesions on T2-weighted and fluid-attenuated inversion recovery (FLAIR) images were graded from Grade 0 to 3: Grade 0=absent; Grade 1=mild changes in the deep white matter (punctate foci); Grade 2=moderate changes in the deep white matter (beginning confluence of foci); and Grade 3=severe form of white matter lesions (extensive large confluence). (Lower): Representative DWL progression (DWLP) subject (Group DWLP) showing progression on the MRI-based Fazekas classification. A DWLP case (64-year-old male in 2003) shows progression on T2-weighted and FLAIR images in 2008 (corresponding to Grade 2=beginning confluence of foci) compared with those from 2003 (corresponding to Grade 1=punctate foci). These white matter changes are classified according to the Fazekas classification.

(16 subjects) comprised those with atherothrombotic
4 infarctions (Table 1). Lacunar infarction was defined as a small infarction of less than 20 mm in diameter according to previous proposals.[14,15] Atherosclerotic infarction was defined as severe stenosis (>50%) or occlusion of the intracranial or extracranial large vessel on the affected side. These subjects had remained free of symptomatic recurrence over the previous 5 years at the outpatient clinic and had no changes on repeated brain MRI in 2003 and 2008 in the same medical survey.

Regarding the evaluation of covariates, to determine which of the main parameters have a significant effect on DWLs and their progression, the associations of various clinical vascular risk factors, including TGF-beta1 levels, which were not recognized as important at the time of the baseline survey, were examined carefully in the recruited groups. The classical risk factors examined consisted of age, sex, hypertension (systolic blood pressure $\geq$140 mmHg, diastolic blood pressure $\geq$90 mmHg, or a history of hypertension plus antihypertensive medication), diabetes mellitus (fasting glucose $\geq$126 mg/dl or treatment with insulin or oral hypoglycemics), hyperlipidemia (triglycerides $\geq$150 mg/dl or high-density lipoprotein cholesterol<40 mg/dl or treatment with lipid-reducing drugs), current smoking (smoking cigarettes in the last 30 days), and the presence of preceding ischemic heart disease. These lifestyle factors or behaviors were investigated using a self-completed questionnaire. Routine examinations of peripheral blood and biochemistry were performed as part of the health-screening check-up. To focus on the changes in TGF-beta1 levels and the clinical characteristics of all subjects, serum samples were collected from each group for the measurement of this candidate inflammatory cytokine. Its concentration was measured using the Multi-Cytokine Detection System according to the manufacturer's protocol as described previously.[16]

Evaluation of Brain MRI

Brain MRI scans were obtained from all subjects using a 1.5-T scanner (1.5-T, EPIOS; Shimazu, Inc., Kyoto, Japan), and these were evaluated blindly by two trained, board-certified neurologists (N.K. and T.M.) and one radiologist (K.Y.). The routine protocol consisted of T1-weighted images (repetition time [TR], 611 ms; echo time [TE], 13 ms), T2-weighted images (TR, 4431 ms; TE, 100 ms), and fluid-attenuated inversion recovery (FLAIR) images (delay time, 2200 ms; TR, 8000 ms; TE, 100 ms). Each transverse image was obtained using a 5-mm section thickness. T2-weighted and FLAIR images were used to evaluate the DWLs, which were graded semi-quantitatively from Grade 0 (none) to 3 (severe) according to the Fazekas classification.[17] Their severity was assessed based on the presence of hyperintense lesions in the deep white matter: Grade 0=absent; Grade 1=punctate foci; Grade 2=confluent foci; and Grade 3=extensive confluent foci (Fig. 1). Periventricular hyperintensity was also defined according to the de Groot classification.[18] The examiners discussed the findings to obtain a consensus in cases of discordant MRI evaluations, which were limited to a few cases.

Regarding the 35 subjects with DWL findings in this survey, those with DWLs from Grade 2 to 3 and with no progression in the grade level were assigned to Group DWL. Group DWLP included subjects exhibiting progression, which was defined as an increase in one or more grade level or an apparent worsening of Grade 3. A representative Group DWLP subject is shown in Fig. 1.

The results were compared among the groups, and statistical analysis was performed using the Mann-Whitney *U*-test, with $P<0.05$ considered significant. The classical vascular risk factors and TGF-beta1 levels for Groups DWL and DWLP and their 95% confidence intervals (CIs) were calculated by logistic regression.

Results

The TGF-beta1 levels were 13.8 ng/ml in Group C, 17.6 ng/ml in Group L, and 19.4 ng/ml in Group A, with no significant difference between these groups,

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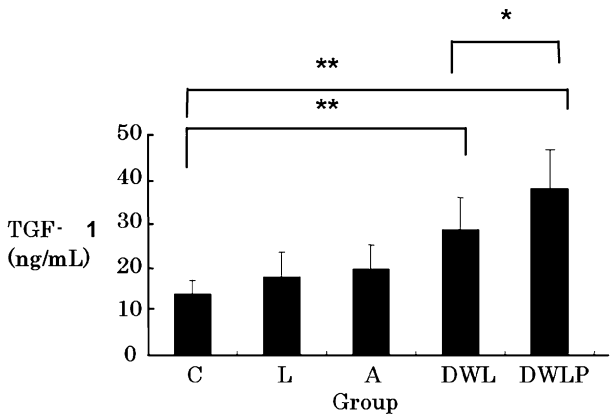

Figure 2 Comparison of TGF-beta1 levels in the groups. TGF-beta1 levels are significantly higher in Groups DWL and DWLP than in Group C, and significantly higher in Group DWLP than in Group DWL. *$P<0.05$ versus Group DWL; **$P<0.01$ versus Group C.

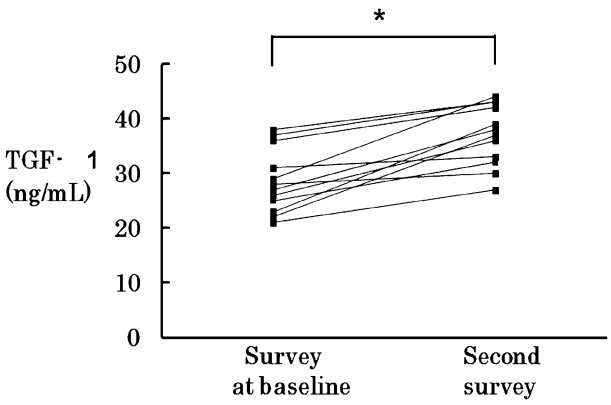

Figure 3 Line graph regarding the change in TGF-beta1 levels in Group DWLP between the survey at baseline and the second follow-up survey. A significant increase in TGF-beta1 levels was observed over the 5-year follow-up period (*$P<0.05$).

but they were significantly higher in Groups DWL (28.2 ng/ml) and DWLP (37.6 ng/ml) than in Group C ($P<0.01$) (Fig. 2). Among all the assigned groups, TGF-beta1 levels were found to be the highest in Group DWLP; moreover, TGF-beta1 levels were significantly higher in Group DWLP than in Group DWL ($P<0.05$). With respect to the existence of periventricular hyperintensity, no significant association with TGF-beta1 levels was observed among the groups.

As summarized in Table 1, there was no significant difference in age or sex among the groups. The percentage of patients with hypertension and hyperlipidemia was significantly higher in Groups L, A, DWL, and DWLP than in Group C. Of all of the groups, Group DWLP had the highest incidence of diabetes mellitus. The classical vascular risk factors (hypertension, hyperlipidemia, and diabetes mellitus) and periventricular hyperintensity were significantly associated with Groups DWL and DWLP on univariate logistic regression analysis. As TGF-beta1 levels were elevated in Groups DWL and DWLP, the statistical significance of this elevation was examined more closely. Using 35 ng/ml (90th percentile values of Group DWL) as a cutoff value of TGF-beta1 for a potential diagnosis of DWLP, elevated TGF-beta1 levels were found to be a significant independent factor in Group DWLP compared with Group C ($P<0.05$) (crude odds ratio [OR]=1.72). There was no similar tendency in Group DWLP compared with Group C (crude OR=1.03). Compared with Group C, elevated TGF-beta1 levels (>35 ng/ml) remained significant for DWLP following multiple adjustments (age, sex, hypertension, hyperlipidemia, diabetes mellitus, ischemic heart disease, periventricular hyperintensity, and smoking) (OR=1.37; 95% CI: 1.19–3.61). The sensitivity was 72.7%, and the specificity was 80.0%, with a positive predictive value of 64.0% in Group DWLP. Logistic regression analysis between Groups DWL and DWLP indicated that elevated TGF-beta1 levels were also a significant independent factor in Group DWLP compared with Group DWL ($P<0.05$) (OR=1.12; 95% CI: 1.04–2.31).

When we evaluated the change in TGF-beta1 levels in Group DWLP between baseline and the second survey, we confirmed the statistical tendency for the increase in TGF-beta1 levels over the 5-year follow-up period ($P<0.05$) (Fig. 3). There was no case showing no change or a decrease in TGF-beta1 levels during the 5-year follow-up period, suggesting that the increase in TGF-beta1 levels could be used to evaluate the progression of DWLs as a new surrogate marker or it may be a trigger that promotes the formation of DWLs.

Discussion

The mechanisms underlying the development of DWLs and their aggravation (DWLP) and the identity of surrogate markers have not yet been clarified. However, vascular inflammation is known to be crucial in the progression of atherosclerosis.[19] Previously, we reported the presence of inflammatory cytokine markers in recurrent cerebral infarction, suggesting their clinical usefulness for the prevention of recurrence.[16] Transforming growth factor-beta, which is evaluated in the present study, reportedly promotes vascular ischemia or intracranial fibrosis.[20] Recently, studies using TGF-beta1 transgenic mice have also shown enhanced TGF-beta expression around intracranial microvessels.[21]

In the present study, TGF-beta1 levels are higher in Groups DWL and DWLP than in the other groups, and more interestingly, they are significantly higher in Group DWLP than in Group DWL. The OR for the presence of elevated TGF-beta1 levels in Group DWLP is statistically significant, even after it

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was adjusted for the major vascular risk factors, including the presence of diabetes, which is suggested to have a close association with plasma TGF-beta1 levels.[22] Taking all the previous reports[4–9] and our data together, it was nevertheless possible to observe, in this pilot study for a large DWL study, that elevated TGF-beta1 levels may be important. These results suggest that TGF-beta1 might play certain roles in promoting the formation of cerebral white matter lesions.

The data were obtained from a single center enrolling consecutive subjects who responded to a random mailing recruitment approach in order to minimize the effect of individual selection bias. Although random mailing was used to recruit the subjects, a small amount of selection bias was unavoidable. Even though the number of DWL and DWLP events, the small sample size, and the relatively short follow-up period meant that this study was insufficiently powered for the analysis of DWL and DWLP events, a correlation between DWLP and elevated TGF-beta1 levels was observed.

Regarding TGF-beta1, Hara *et al.*[4] reported that TGF-beta1 was overexpressed in the extracellular matrix of cerebral small vessels, the choroid plexus, and the brain tissue. There have also been several reports that the overexpression of TGF-beta1 in peripheral blood can also be observed in the matrix of atherosclerotic artery walls themselves or the renin-angiotensin system of the kidney in humans.[23,24] Thus, it is also necessary to evaluate the origin of TGF-beta1 in the above-mentioned organs, related tissues, or cerebrospinal fluid.

The present study has two major limitations. One is its relatively small sample size, resulting in insufficient statistical power to assess this hypothesis with a high degree of confidence. To evaluate the diagnostic value of TGF-beta1, a large-scale cohort study is needed. The other limitation is that the data were obtained from a single center enrolling consecutive subjects who responded to a random mailing recruitment approach in order to minimize the effect of individual selection bias. Although random mailing was used to recruit the subjects, a small amount of selection bias was unavoidable. In summary, the limited numbers of DWL and DWLP events, the small sample size, the individual selection bias, and the relatively short follow-up period meant that this study was insufficiently powered for the analysis of DWL and DWLP events and should be considered when we assess the correlation between DWLP and elevated TGF-beta1 levels.

A possible mechanism underlying this relationship between TGF-beta1 elevation and DWLP is the participation of chronic kidney disease (CKD). Previously, we reported that DWLs and related cognitive impairment are associated with CKD.[12] Conversely, TGF-beta1 is known to aggravate systemic arteriosclerosis and subsequent hypertensive changes through chronic renal dysfunction.[25,26] Therefore, kidney dysfunction may be a pathological condition that supports this hypothesis for the role of TGF-beta1 in DWLP. Thus, the next step in this research would be to focus on these organ-specific diseases to identify the pathological effects on or the relationships of TGF-beta1 with DWLP.

There are some reports showing that TGF-beta1 has a role in aneurysm formation, including cases of Marfan syndrome, by weakening the blood vessel wall and compromising angiogenesis.[27,28] We also contemplated the potential role of the elevated TGF-beta1 levels in Group DWLP in the progression of intracranial white matter lesions. These elevated levels are thought to reflect intracranial arterial wall weakening and the breakdown of the blood–brain barrier, in a similar mechanism to that underlying aneurysm formation. Therefore, TGF-beta1 is an interesting surrogate candidate marker for the progression of white matter lesions, which might reflect pathological changes such as *de novo* angiogenesis.

Recently, Sorice and Folli[29] reported that statins and peroxisome proliferator-activated receptor-gamma agonists could represent a useful novel adjunctive therapy for reducing the action of TGF-beta in the molecular pathogenesis of aneurysms and improving matrix repair in atherosclerosis. From that point of view, if we measure the levels of TGF-beta1 in patients at high risk of recurrent stroke annually, increased TGF-beta1 levels may be a suitable surrogate blood marker to survey the gradual change in white matter lesions from the initial stage. This implies that we may decrease the occurrence of recurrent stroke or vascular dementia by monitoring these patients carefully in the future.

Our eventual research strategy is to evaluate all the relevant measures, including MRI findings, in these groups in 2013, after a 10-year follow-up, as well as to clarify the role of various TGF-related inflammatory mediators. The present findings on the impact of intracranial atherosclerosis on TGF-beta1 serum levels are the first to be published, but a large-scale cohort study is necessary to clarify the direct relationship between DWLP and the changes in the levels of the TGF-beta family to confirm TGF-beta1 as a direct novel risk factor for DWLs, including genetic factors such as related single nucleotide polymorphisms.

In conclusion, increased TGF-beta1 levels may be an important factor for the pathogenesis of DWLs, directly or indirectly, by affecting the mechanism of DWL formation and their progression.

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Disclosure

None.

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