Decreased plasma decorin levels following acute ischemic stroke: Correlation with MMP-2 and differential expression in TOAST subtypes

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Abstract. Accumulating evidence suggests that extracellular matrix (ECM) remodeling plays a significant role following acute ischemic stroke (AIS). Decorin (DCN) is a well-recognized molecule present in the ECM; however, the role of DCN in AIS remains unknown. The present study aimed to investigate whether plasma concentrations of DCN are altered in patients following an AIS and whether they are correlated with matrix metalloproteinase-2 (MMP-2) levels and other laboratory and clinical variables. Plasma concentrations of DCN were assessed in 102 patients with AIS (less than 7 days) and 120 control subjects using ELISA assays. The correlation between DCN concentrations and MMP-2 levels, Trial of Org 10172 in Acute Stroke Treatment (TOAST) subtypes, stroke severity and risk factors were evaluated. The expression of DCN was significantly decreased in patients with AIS (P<0.001), particularly in the large-artery atherosclerosis (LAAS) group. The levels of DCN were positively correlated with MMP-2 (R=0.332; P<0.001), thus MMP-2 is an independent predictor of DCN concentration (P<0.001). DCN levels below 8,500 pg/ml had sensitivity and specificity values of AIS of 79.4 and 62.8%, respectively and DCN below 8,500 pg/ml was associated with AIS (OR=4.8; 95% CI: 2.1-11.1; P<0.001) following adjustment for potential confounders. In conclusion, for the first time, a reduction in DCN was detected in patients following AIS and these altered plasma concentrations were correlated with MMP-2. Larger studies are required to further investigate whether DCN is involved in the pathogenesis of ischemic stroke.

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Abbreviations: AF, atrial fibrillation; AIS, acute ischemic stroke; ANOVA, analysis of variance; APTT, activated partial thromboplastin time; AUC, area under the curve; CEI, cardioembolic infarcts; CT, computed tomography; DBP, diastolic blood pressure; DM, diabetes mellitus; ECM, extracellular matrix; EGF-R, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; FDP, fibrinogen degradation products; FIB, fibrinogen; GAG, glycosaminoglycan; HDL, high-density lipoprotein; IGF-1R, insulin-like growth factor-1 receptor; IHD, ischemic heart disease; IL, interleukin; LAAS, large-artery atherosclerosis; LAC, lacunar infarct; LDL, low-density lipoprotein; MMP, matrix metalloproteinase; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; ODE, stroke of other determined etiology; PDGF, platelet-derived growth factor; PLT, platelet; PT, prothrombin time; RBC, red blood cell; SBP, systolic blood pressure; SD, standard deviation; TC, total cholesterol; TG, triglyceride; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor-α; TOAST, Trial of ORG10172 in Acute Stroke Treatment; UDE, stroke of undetermined etiology; WBC, white blood cell

Key words: decorin, matrix metalloproteinase-2, stroke, extracellular matrix

Introduction

Acute ischemic stroke (AIS) is a common cause of morbidity and mortality in industrialized countries. According to the World Health Organization, stroke affects approximately 5.8 million individuals each year worldwide (1). Stroke may trigger an inflammatory reaction that lasts several months. It has been reported that the suppression of inflammation with a variety of drugs reduces infarct volume and improves clinical outcomes in animal models of stroke (2). Extracellular matrix (ECM) remodeling, effected by a group of significant inflammatory molecules, including matrix metalloproteinases (MMPs), may be a key factor in the development of inflammation in the central nervous system. Proteoglycans are thought to cause matrix remodeling and modulate the activity of growth factors and cytokines (3).

Decorin (DCN) is a small proteoglycan that consists of a single glycosaminoglycan side chain and is linked to a core protein containing leucine-rich repeats of 24 amino acids. It is present in the ECM of a variety of tissues and cell types (4).

During the past decade, accumulating evidence has suggested that DCN interacts with a variety of biological molecules, including type I collagen, fibronectin, thrombospondin, epidermal growth factor receptors (EGF-Rs), insulin-like growth factor-1 receptors (IGF-1Rs), platelet-derived growth factor (PDGF), complement C1q and MMPs, particularly MMP-2 and MMP-9 (5-8). These factors are involved in matrix assembly and may be involved in the regulation of fundamental biological functions, including cell attachment, migration and proliferation (9-12). It has also been reported that DCN is involved in the regulation of collagen fibrillogenesis, ECM remolding or deposition and cancer cell growth. However, no studies have investigated the expression of DCN in patients with AIS and in particular the correlation between DCN and MMP-2 has not been explored.

The present study aimed to investigate the levels of DCN and MMP-2 in normal volunteers as well as in subjects with AIS. Furthermore, the correlations between plasma DCN and MMP-2 and other varying clinical factors in the subjects were analyzed and the role of DCN as a biomarker of risk for AIS was explored.

Subjects and methods

Subjects and assessment. Patients with AIS were recruited from the Department of Neurology, Changhai Hospital, Second Military Medical University, Shanghai, between April 2010 and May 2011. A total of 120 volunteers matched for age, gender and cardiovascular risk factors, including hypertension, diabetes mellitus (DM), hypercholesterolemia and heart disease, were included as controls. Diagnosis of AIS was defined by focal neurological signs or symptoms of potential vascular origin that persisted for >24 h and confirmed using brain computed tomography (CT) and/or magnetic resonance imaging (MRI) within 7 days following the onset of stroke according to The International Classification of Diseases (9th Revision) (13). Exclusion criteria for the two groups were: (a) infectious disease or traumatic injury in the previous month; (b) myocardial infarction in the previous year; (c) severe renal and liver failure; (d) history of any chronic inflammatory disease; (e) history of cancer. Smoking history and history of hypertension, DM, hypercholesterolemia, or any heart disease were recorded.

In this study, stroke subtype was classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria (14): (a) large-artery atherosclerosis (LAAS); (b) cardioembolic infarct (CEI); (c) lacunar infarct (LAC); (d) stroke of other determined etiology (ODE); (e) stroke of undetermined etiology (UDE).

Stroke severity was scored using the National Institutes of Health Stroke Scale (NIHSS) on admission and at 72 h (15).

This project was performed according to the principles of the Declaration of Helsinki and approved by the local ethics commission. All patients gave informed consent. All data were evaluated by a trained neurologist and a researcher who were blinded to each other's assessment.

Laboratory tests and DCN measurements. The blood samples from all patients were collected by laboratory specialists on the first day of admission. Routine laboratory tests, including red blood cell (RBC), white blood cell (WBC), platelet (PLT), plasma glucose, fibrinogen degradation products (FDP), fibrinogen (FIB), prothrombin time (PT), activated partial thromboplastin time (APTT), triglyceride (TG), total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were determined using an automatic analyzer (Hitachi 7060, Tokyo, Japan).

Blood samples for DCN and MMP-2 were collected into chilled tubes containing EDTA-Na_2 (1 mg/ml blood) and centrifuged at 4,000 rpm at 4˚C for 10 min. The supernatants were decanted and frozen at -80˚C until assayed. DCN was measured using enzyme-linked immunosorbent assay (ELISA) with Raybio human DCN ELISA kits (Raybiotech, Norcross, GA, USA) according to the manufacturer's instructions. MMP-2 was treated using R&D Human MMP-2 Immunoassay kit (R&D Systems, Minneapolis, MN, USA) following the manual procedure. Intra- and inter-assay coefficients of variation for the two were <10%.

Statistical analyses. All statistical analyses were performed using SPSS 17.0 software. Values are expressed as mean ± SD or, in the case of non-normally distributed data, as median and interquartile range. Data that were normally distributed were analyzed using a Student's t-test (for two groups) or one-way analysis of variance (ANOVA; for three groups). Non-normally distributed data were analyzed using the nonparametric Mann-Whitney U test (for two groups) or Kruskal-Wallis test (for three groups). Pearson's correlation coefficient was calculated to evaluate a possible correlation in continuous variables. Multiple stepwise linear regression analyses were performed to identify independent determinants of plasma DCN concentration. Logistic regression analyses were used to assess whether stroke was related to traditional atherosclerotic risk factors and DCN. For the assessment of the accuracy of the parameter of DCN in discriminating between stroke patients and controls, receiver operating characteristic curve (ROC) analyses were performed. Data were considered to be statistically significant when $P<0.05$.

Results

Plasma levels of DCN and MMP-2 are decreased in AIS patients. The study population consisted of 102 AIS patients (71 men; 31 women) and 120 controls. Demographic and clinical characteristics are shown in Table I. The vascular risk factors, including ischemic heart disease (IHD), hypertension, atrial fibrillation (AF), DM, hyperlipidemia, smoking and alcohol consumption did not differ significantly between the groups. No significant differences in PLT, PT, APTT, D-dimer, serum triglycerides (TGs), HDL and total cholesterol (TC) were observed between the two groups (Table I).

Plasma levels of DCN were significantly lower in AIS patients compared with individuals in the control group ($P<0.001$, Table I, Fig. 1). Similarly, plasma levels of MMP-2 were significantly lower in patients than in the controls ($P<0.001$, Table I, Fig. 1). The plasma DCN levels were positively correlated with MMP-2 ($R=0.332$; $P<0.001$).

Plasma DCN levels are lowest in patients with LAAS. Patients were divided into five subgroups according to the criteria

Table I. Clinical characteristics and laboratory parameters of patients with and without stroke.

Demographic	AIS (n=102)	Control (n=120)	P-value
Male, n (%)	71 (69.6)	76 (63.3)	0.393
Age, years	61.3±14.4	61.6±12.2	0.168
Stroke subtype of etiology (TOAST), n (%)			
Cardioembolic infarcts	8		
Large-artery atherosclerosis	59		
Lacunar infarct	8		
Stroke of other determined etiology	7		
Stroke of undetermined etiology	20		
Risk factor, n (%)			
History of ischemic heart disease	9 (8.8)	15 (12.5)	0.379
Hypertension	64 (62.7)	77 (64.2)	0.274
Diabetes mellitus	23 (22.5)	27 (22.5)	0.993
Atrial fibrillation	10 (9.8)	13 (10.8)	0.802
Hyperlipidemia	7 (6.9)	10 (8.3)	0.681
Cigarette smoking, n (%)	56 (54.9)	51 (42.5)	0.193
Never	46 (45.1)	69 (57.5)	
Former	23 (22.5)	21 (17.5)	
Current	33 (32.4)	30 (25)	
Alcohol intake, n (%)	55 (53.9)	60 (50)	0.801
Non-drinker, n	47	60	
Former-drinker, n	34	42	
Drinker, n	21	18	
Laboratory results			
RBC ($x10^{12}$/l)	4.6±0.6	4.3±0.4	0.002
WBC ($x10^{9}$/l)	7.4±2.3	6.7±1.3	0.006
PLT ($x10^{9}$/l)	206.5±47.8	202.2±37.0	0.462
PT (s)	13.9±5.0	13.1±1.6	0.147
APTT (s)	37.9±9.4	37.7±3.8	0.865
FIB (g/l)	3.56±1.29	3.16±0.44	0.004
D-dimer (μg/ml)	1.09±2.36	0.67±0.50	0.60
FDP (μg/ml)	5.89±7.41	2.97±1.31	<0.001
LDL (mmol/l)	3.08±1.20	2.56±0.51	<0.001
HDL (mmol/l)	1.01±0.22	1.00±0.19	0.767
TG (mmol/l)	1.58±0.66	1.65±0.64	0.47
TC (mmol/l)	4.70±1.17	4.45±0.74	0.063
Plasma glucose (mmol/l)	6.2±2.1	5.1±1.4	<0.001
Decorin (pg/ml)	7094.8±2150.67	8950.04±1267.65	<0.001
MMP-2 (ng/ml)	132.37±37.2	185.92±33.94	<0.001
Vital signs at admission			
Body temperature (˚C)	36.5±0.5	36.6±0.5	0.078
SBP (mmHg)	139.8±20.0	129.6±13.4	<0.001
DBP (mmHg)	84.8±11.0	75.4±9.5	0.099
Heart rate (/min)	77.6±6.5	74.2±8.9	<0.001

Continuous variables are expressed as mean ± SD. APTT, activated partial thromboplastin time; DBP, diastolic blood pressure; FDP, fibrinogen degradation products; FIB, fibrinogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PLT, platelet; PT, prothrombin time; RBC, red blood cell; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; TOAST, Trial of ORG10172 in Acute Stroke Treatment; WBC, white blood cell; AIS, acute ischemic stroke.

used in TOAST. The five stroke subgroups were as follows: CEI, n=8; LAAS, n=59; LAC, n=8; ODE, n=7; UDE, n=20. The DCN levels were 9183.2±1892.65, 6640.63±2050.75, 7877.87±2022.54, 6966.03±2104.68 and 7331.09±2150.67 in groups CEI, LAAS, LAC, ODE and UDE, respectively. The lowest DCN levels were identified in patients with LAAS. Patients with CEI demonstrated the highest DCN levels among the five groups. Fig. 3 shows that DCN levels were signifi-

Table II. Pearson correlation between DCN and clinical laboratory parameters.

Patient characteristics	DCN	
	R	P-value
Age	-0.14	0.830 (NS)
Systolic blood pressure	-0.188	0.005[b]
Diastolic blood pressure	-0.180	0.007[b]
Ischemic heart disease	-0.026	0.705 (NS)
Atril fibrillation	0.181	0.007[b]
Plasma glucose	-0.159	0.017[a]
Fibrinogen	-0.045	0.509 (NS)
FDP	-0.199	0.003[b]
D-dimer	-0.122	0.069 (NS)
Total cholesterol	-0.084	0.213 (NS)
Triglyceride	0.059	0.382 (NS)
High-density lipoprotein	-0.058	0.391 (NS)
Low-density lipoprotein	-0.154	0.022[a]
NIHSS	0.12	0.906 (NS)
MMP-2	0.332	<0.001[b]

DCN, decorin; FDP, fibrinogen degradation products; MMP-2, matrix metalloproteinase-2; NS, not significant; NIHSS, National Institutes of Health Stroke Scale. [a]P<0.05; [b]P<0.01.

Table III. Cut-off values of DCN in AIS and controls.

Cut-off (pg/ml)	Sensitivity (%)	Specificity (%)
9,000	79.4	47.5
8,500	73.5	60.0
8,000	69.6	78.3
8,500[a]	79.4	62.8
8,500[b]	79.4	39.0

[a]Controls (n=43) were individuals without risk factors; [b]Controls (n=77) were individuals with risk factors; DCN, decorin; AIS, acute ischemic stroke.

cantly lower in patients with LAAS, LAC, ODE and UDE than those in controls (P<0.001, P=0.028, P<0.001, P<0.001, respectively). However, no difference was noted in DCN levels in patients with CEI as compared with controls.

DCN levels <8,500 pg/ml are associated with AIS. Correlation analyses (Table II) revealed a positive correlation between DCN levels and MMP-2 (R=0.332; P<0.001) and AF (R=0.181; P=0.007). A negative correlation was revealed between DCN levels and systolic blood pressure (R=-0.188; P<0.001), diastolic blood pressure (R=-0.180; P=0.005), plasma glucose (R=-0.159; P=0.017), FDP (R=-0.199; P=0.003) and LDL (R=-0.154; P=0.022). However there was no correlation between DCN levels and NIHSS (R=0.12; P=0.906). Multiple stepwise linear regression analyses revealed that MMP-2 and AF were significant independent determinants of DCN

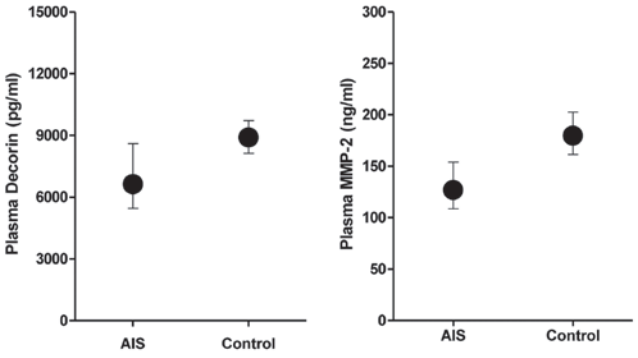

Figure 1. Plasma decorin and MMP-2 levels in subjects with AIS and controls. AIS, acute ischemic stroke.

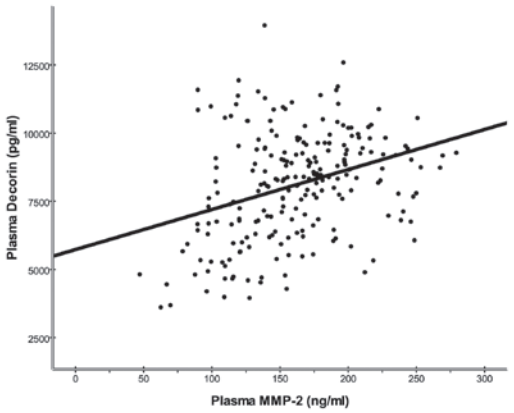

Figure 2. Positive correlation between decorin and MMP-2. MMP-2, matrix metalloproteinase-2.

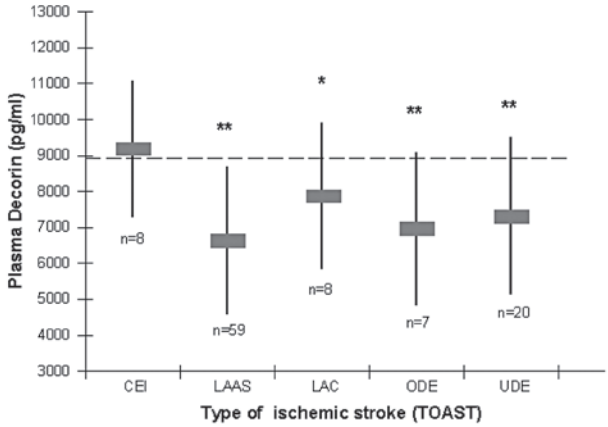

Figure 3. Mean values with 95% CI of plasma decorin levels in each subgroup of ischemia stroke. The dotted line represents the level of decorin in the control group. CEI, cardioembolic infarct; LAAS, large-artery atherosclerosis; LAC, lacunar infarct; ODE, stroke of other determined etiology; UDE, stroke of undetermned etiology.

(β=0.341, P<0.001; β=0.197, P=0.002, respectively), when the vascular risk factors, including age, gender, hypertension, DM, smoking, alcohol, TC, LDL and HDL were taken into account as independent variables. Following adjustment for smoking, alcohol, DM and hypertension, logistic regression analyses revealed that DCN levels <8,500 pg/ml were associated with AIS (OR=4.8; 95% CI: 2.1-11.1; P<0.001).

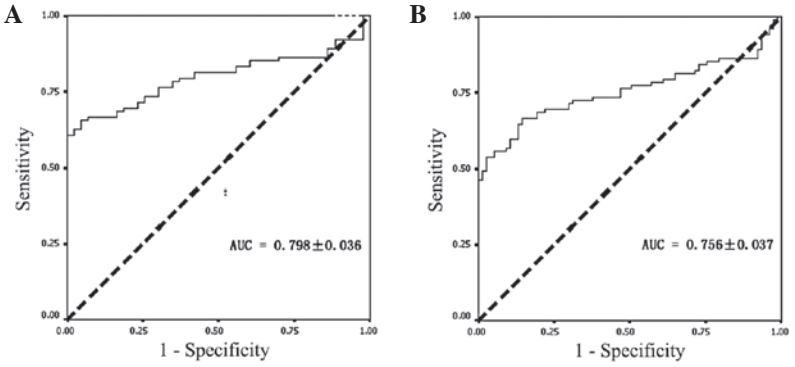

Figure 4. Receiver operating characteristic (ROC) curves for plasma DCN values in subjects with known vascular risk factors (A) and those without (B). AUC, area under the ROC curve; DCN, decorin.

Plasma DCN (8,500 pg/ml) has a sensitivity and specificity for AIS of 79.4 and 62.8%, respectively. To define the optimal cut-off value for DCN levels, an ROC curve was constructed (Fig. 2). The controls (n=120) were divided into the patients with known vascular risk factors (n=77) and those without (n=43) and three cut-off values were calculated (8,000, 8,500 and 9,000 pg/ml) which provided various accuracies (Table III). The cut-off value used for DCN levels was 8,500 pg/ml and the area under the curve (AUC) for DCN was 0.798 (95% CI: 0.73-0.87; P<0.001) in the patients with AIS and controls without known vascular risk factors (n=43). The sensitivity and specificity of plasma DCN (8,500 pg/ml) for AIS were 79.4 and 62.8%, respectively.

Discussion

DCN is a ubiquitous small extracellular proteoglycan. It is a composite molecule, 100 kDa in size, with a protein core and attached glycosaminoglycans (GAGs) (16). It was cloned from a human embryonic fibroblast line and named PG40 due to its protein core (40 kDa) (4). Previous studies have suggested that DCN is involved in the regulation of collagen fibrillogenesis, ECM remolding, tumor growth and metastasis, angiogenesis, renal and pulmonary fibrosis, muscular dystrophy, wound healing and myocardial infarction (17-20). Although previous studies have furthered our understanding of DCN in various pathological processes, the role of DCN in patients with AIS has not been investigated. The present study examined plasma DCN and MMP-2 in subjects with AIS. For the first time, this study revealed that there are lower plasma levels of DCN in subjects with AIS compared with those in controls. Decreased DCN levels (<8,500 pg/ml) had 79.4% sensitivity and 62.8% specificity for AIS, therefore this cut-off point might be a useful indicator for ischemic stroke.

MMPs underlie a tight regulatory process resulting in the equilibrium between the synthesis and degradation of ECM components (21-23). This equilibrium may be disturbed following stroke, leading to the decreased synthesis of MMP-2. However, conflicting data have reported an increase (24) or decrease (25) in plasma levels following ischemic stroke. The observed decrease in MMP-2 levels following AIS in the current study may be explained pathophysiologically as a failure to detect circulating MMP-2 due to an accelerated MMP turnover, binding of MMP-2 to damaged tissue or increased utilization of MMP-2 for local matrix remodelling (26). In the current study, plasma DCN levels were positively correlated with MMP-2. Therefore, these results are in agreement with those of a previous study, in which a recombinant adenovirus vector containing a DCN gene transfection to rat mesangial and tubular cells led to the repressed expression of transforming growth factor-β (TGF-β) and collagen type IV, whilst it promoted the expression of MMP-2 and -9 (27). Similarly, Al Haj Zen *et al* (28) demonstrated that DCN may affect the production of metalloproteinases and cytokines through the adenovirus-mediated overexpression of DCN in the human gingival fibroblasts. In this study, the authors also demonstrated that DCN infection resulted in the decreased expression of MMP-1, MMP-3, TGF-β and IL-1β. However, the levels of MMP-2, tissue inhibitor of metalloproteinase -2 (TIMP-2) and IL-4 were markedly increased. Consistent with previous studies, the results of the current study also suggest that plasma DCN levels are involved in the development of ischemic stroke by interacting with MMP-2.

Atherosclerosis, an underlying cause of a large proportion of strokes, is a multifactorial process which synergistically induces oxidative stress and a chronic inflammatory state (29). Lipids, particularly cholesterol transported in circulating LDL particles, are key factors that are associated with the formation of the atherosclerotic plaque. In a previous study, the authors reported a reduction of atherosclerosis development in the animal model of ApoE-/- mice treated using adenoviruses containing the human DCN gene (3). The secreted DCN formed complexes with 40% of plasma TGF-β1 of ApoE-/- mice, leading to a reduction in plasma TGF-β1. The overexpression of DCN was accompanied by a reduction in plaque macrophage content. Atherosclerosis protection is achieved since transient DCN overexpression changes the plaque phenotype to that of lower inflammation. Similarly, in the current study, the level of DCN was the lowest in the LAAS group and it was negatively correlated with LDL-C. This result is consistent with previous studies and suggests that plasma DCN levels are involved in the development of atherosclerosis. The correlation between DCN and NIHSS was also evaluated. NIHSS is a reliable and commonly used score for clinical assessment of the severity of stroke. However, no correlation was identified between DCN levels and NIHSS in the present study. This might be due to the small sample size. Therefore, further studies are required to clarify the correlation between the DCN and NIHSS.

During AIS, the brain initiates a complex cascade of ischemic events at various levels. Excitotoxicity, oxidative stress, blood-brain barrier dysfunction, inflammation and ECM remolding are the most significant pathophysiological processes involved in this cascade (30-32). Cytokines, including IL-1, IL-6, tumor necrosis factor-α (TNF-α) and TGF-β are produced by a variety of activated cell types and are significant mediators of stroke induced inflammation that may contribute to the progression of cerebral infarction (33-35). Yamaguchi *et al* (36) demonstrated that DCN is a natural inhibitor of transforming growth factor-β1 (TGF-β1). Al Haj Zen *et al* (3) reported that DCN reduces inflammation by downregulating TGF-β and IL-1β expression through adenovirus-mediated overexpression of DCN in the human gingival fibroblasts. These studies indicate that inflammatory cytokines explain the change of DCN expression during stroke.

There are several limitations to the present study. First, the sample size was relatively small. Second, there were no long-term follow-up results. Thus, further studies are required to confirm these preliminary results.

In conclusion, the present study is the first to report that plasma DCN levels are decreased in patients with AIS, particularly in the LAAS group, and that the level of DCN is positively correlated with MMP-2 levels. Of note, decreased plasma DCN concentrations (<8,500 pg/ml) are associated with increased risk for ischemic stroke. Our findings indicate that DCN is involved in the pathogenesis of ischemic stroke and that a reduction of plasma DCN may be a useful indicator for ischemic stroke.

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