Serum Thrombomodulin in Myeloperoxidase-Antineutrophil Cytoplasmic Antibody (MPO-ANCA) -Associated Vasculitis.

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Background and Aims : Although serum or plasma thrombomodulin (TM) was reported to be increased in various diseases with vascular injuries, blood TM was also shown to be influenced by renal function. In this study, we determined whether serum TM (sTM) could be a marker of vascular injury in patients with myeloperoxidase-antineutrophil cytoplasmic antibody (MPO-ANCA) -associated vasculitis, in which kidneys are often affected. **Methods :** Thirteen patients with MPO-ANCA-associated vasculitis were investigated and 43 patients with chronic glomerulonephritis (CGN) served as a control. sTM was measured by one-step sandwich enzyme immunoassay method. **Results :** A significant positive correlation was found between sTM and serum creatinine (sCr) ($sTM = 2.24 \times sCr + 2.16$, $r = 0.91$, $P < 0.001$) in patients with CGN. In order to correct sTM for renal function, we calculated corrected sTM according to the following formula: $\text{corrected sTM (\%)} = [\text{measured sTM} / \text{expected sTM at the measured sCr}] \times 100 = [sTM / (2.24 \times sCr + 2.16)] \times 100$. Patients with MPO-ANCA-associated vasculitis tended to have lower levels of corrected sTM before treatment compared to patients with CGN, but not significantly ($86.8 \pm 23.2\%$ vs. $98.7 \pm 18.3\%$, ANCA vs. CGN, $P = 0.12$). Corrected sTM was increased significantly within 1 month after the beginning of treatment ($145.7 \pm 46.9\%$, $P = 0.008$, vs. before treatment in each patient). In remission, corrected sTM returned to the same levels of CGN ($104.5 \pm 38.6\%$, $P = 0.03$, vs. within 1 month in each patient). **Conclusions :** These results showed that sTM corrected for renal function does not simply reflect disease activity in MPO-ANCA-associated vasculitis. (Kitakanto Med J 2005 ; 55 : 13~18)

Key Words : thrombomodulin, MPO-ANCA, vasculitis

Introduction

Thrombomodulin (TM) is a glycoprotein that exists on the surface of vascular endothelial cells. It plays an important role in protecting clot formation in the vasculature by neutralizing thrombin clotting activity and accelerating the thrombin-catalyzed activation of protein C.^{1,2} When thrombin is complexed with TM in vivo, protein C activation is markedly enhanced and activated protein C, in turn, results in decreased thrombin generation,² thus, TM is considered a natural anticoagulant.

TM is widely distributed throughout the endothelium of arteries, veins, capillaries, lymphatics, and on syncytiotrophoblast of human placenta.³ TM is also

detected in blood and urine in a fragmented soluble form.⁴ This fragmented TM is considered a derived form of injured endothelial cell membranes, as well as proteolytic cleavage of membrane-bound TM.^{5~7} Increased level of serum or plasma TM has been reported in various diseases, in which endothelial cells were injured, such as disseminated intravascular coagulation syndrome, adult respiratory distress syndrome, diabetes mellitus, and systemic lupus erythematosus.^{1,8~16}

Antineutrophil cytoplasmic autoantibodies (ANCA) are serologic markers for various diseases, ranging from multisystem vasculitis, such as Wegener's granulomatosis and polyarteritis nodosa, to renal limited disease (that is, idiopathic crescentic glomer-

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ulonephritis).¹⁷ The autoantibody against proteinase-3 (proteinase-3-antineutrophil cytoplasmic antibody: PR3-ANCA) is usually detected in patients with Wegener's granulomatosis. The autoantibody against myeloperoxidase (myeloperoxidase-antineutrophil cytoplasmic antibody: MPO-ANCA) is typically found in patients with microscopic polyangiitis or necrotizing crescentic glomerulonephritis with or without lung hemorrhage.^{18,19}

In this study, we determined whether serum TM (sTM) could be a marker of vascular injury of MPO-ANCA-associated vasculitis. Blood TM levels are considered to be affected by renal dysfunction, because fragmented blood TM is cleared through the kidneys.^{9,11,16} Kidneys are the main target organs of MPO-ANCA-related vasculitis, thus, renal function often fluctuates in the course of the disease.¹⁹ In this study, we examined the changes of sTM in patients with MPO-ANCA-associated vasculitis before and after treatment and evaluated sTM levels corrected for renal function as a marker of disease activity.

Materials and Methods

Patients with MPO-ANCA-associated vasculitis

Thirteen patients with MPO-ANCA-associated vasculitis were investigated in this study (Table 1). The clinical diagnosis was microscopic polyangiitis in 5 patients, necrotizing crescentic glomerulonephritis with lung hemorrhage in 1, necrotizing crescentic glomerulonephritis with progressive systemic sclerosis and Sjögren syndrome in 1, necrotizing crescentic glomerulonephritis with lung sarcoidosis in 1, necrotizing crescentic glomerulonephritis in 3, rapidly progressive glomerulonephritis (renal biopsy not done) with interstitial pneumonia in 1, and rapidly progressive glomerulonephritis in 1. All patients were positive for MPO-ANCA. Three patients were male and 10

were female. The mean age of patients was 59.9 ± 17.8 (range 18 to 87) years. The averages of MPO-ANCA, C-reactive protein (CRP), and serum creatinine (sCr) before treatment were 547 ± 367 , 10.3 ± 7.9 , and 3.6 ± 1.1 , respectively. All patients were treated with steroids with or without immunosuppressive drugs. Remission was achieved in 9 patients. Six patients died in the course of disease. The serum samples in the complication period that did not relate to primary disease activity, such as bacterial pneumonia and severe heart failure, were eliminated from the study. Informed consent was obtained from the patients.

Patients with chronic glomerulonephritis

The sera of 43 patients with chronic glomerulonephritis (CGN) were examined for correlation between sTM and sCr. The clinical diagnosis was IgA nephropathy in 30 patients, non-IgA mesangial proliferative glomerulonephritis in 2, membranous nephropathy in 2, minimal change nephrotic syndrome in 2, membranoproliferative glomerulonephritis in 1, and chronic glomerulonephritis (renal biopsy not done) in 6. Twenty-three patients were male and 20 were female. The mean age of patients was 45.0 ± 12.5 (range 20 to 69) years. The mean sCr level was 1.7 ± 1.0 (range 0.5 to 4.0)mg/dl. Every CGN patient was treated in our outpatient clinic and had relatively stable renal function. All patients were negative for MPO-ANCA or PR3-ANCA in the CGN group. Informed consent was obtained from the patients.

Assays

sTM levels were measured by the one-step sandwich enzyme immunoassay method developed by Fuji Chemical Industries (Takaoka, Japan).^{20,21} Titers of MPO-ANCA were measured by the enzyme-linked immunosorbent assay method developed by Nissho

Table 1 List of patients with MPO-ANCA associated vasculitis

No.	Age	Sex	Diagnosis	MPO-ANCA (EU/ml)	CRP (mg/dl)	sCr (mg/dl)	Treatment	Prognosis
1	66	M	MPA	710	18.8	4.0	Pulse, P, C	Remission
2	63	M	MPA	902	8.9	5.3	Pulse, P, C	Remission
3	44	F	MPA	1194	16.5	2.6	Pulse, P, PE,	Remission
4	87	F	MPA	757	15.6	2.3	P	Dead (Heart Failure)
5	55	F	MPA	346	5.4	4.3	Pulse, P, C	Remission
6	48	F	NCGN+Pulmonary Hemorrhage	225	22.8	2.5	Pulse, P, C	Remission
7	59	F	NCGN+SSc+SjS	113	15.0	4.0	P, C	Dead (ARDS)
8	51	F	NCGN+Sarcoidosis	212	0.4	5.9	Pulse, P, C	Remission
9	69	F	NCGN	584	18.7	4.0	P	Dead (Heart Failure)
10	18	F	NCGN	845	5.0	2.0	Pulse, P, C	Remission → Dead (Sepsis)
11	63	F	NCGN	63	0.2	3.4	Pulse, P, C	Remission
12	77	M	RPGN+Interstitial Pneumonia	918	4.8	3.6	Pulse, P	Dead (Respiratory Failure)
13	79	F	RPGN	246	2.0	3.4	P	Remission → Dead (Heart Failure)

MPA, microscopic polyangiitis; NCGN, necrotizing crescentic glomerulonephritis; SSc, systemic sclerosis; SjS, Sjögren syndrome; RPGN, rapid progressive glomerulonephritis (renal biopsy not performed); Pulse, methyl prednisolone pulse; P, prednisolone; C, cyclophosphamide; PE, plasma exchange; ARDS, acute respiratory distress syndrome.

Fig.1 Changes of (A) C-reactive protein (CRP), (B) anti-myeloperoxidase antineutrophil cytoplasmic antibody (MPO-ANCA), and (C) serum creatinine (sCr). The data represent one sample or the average of two samples at (I) before treatment, (II) within 1 month after the initiation of treatment, and (III) in remission.

Fig.2 Correlation of serum creatinine (S-Cr) and serum thrombomodulin (sTM). The data represent one sample or the average of two samples at (◇) before treatment, (▲) within 1 month after the initiation of treatment, and (■) in remission, in anti-myeloperoxidase antineutrophil cytoplasmic antibody (MPO-ANCA)-associated vasculitis; and (■) chronic glomerulonephritis.

Fig.3 Corrected serum thrombomodulin (sTM) by renal function. The data represent one sample or the average of two samples at (◇) before treatment, (▲) within 1 month after the initiation of treatment, and (■) in remission, in anti-myeloperoxidase antineutrophil cytoplasmic antibody (MPO-ANCA)-associated vasculitis; and (■) chronic glomerulonephritis.

Industries (Kusazu, Japan).²²

Statistical Analysis

Data were expressed as the mean ± SD. Data were compared by Mann-Whitney's U test or Wilcoxon signed-ranks test. A probability value of $P < 0.05$ was considered significant. All statistical analyses were performed using StatView 5.0 software (Abacus Concepts, Berkeley, CA).

Results

Changes of CRP, MPO-ANCA, and sCr in the course of treatment are shown in Fig.1. Levels of CRP and MPO-ANCA decreased with treatment in all patients with MPO-ANCA-associated vasculitis. Reduced sCr was also observed in most patients, except

1 patient.

Blood TM has been reported to correlate well with renal function, as it is cleared from the kidneys.^{9,11,16} Therefore, we examined the correlation between sCr and sTM in patients with CGN, who had relatively stable renal function. As shown in Fig.2, a strong positive correlation was found between sCr and sTM ($TM = 2.24 \times Cr + 2.16$, $r = 0.91$, $P < 0.001$) in these patients. To exclude the effect of renal function on sTM levels, we evaluated patient sTM using the formula: corrected sTM (%) = [measured sTM/expected sTM at measured sCr level] × 100 = $[sTM / (2.24 \times Cr + 2.16)] \times 100$.

In patients with MPO-ANCA-associated vasculitis, corrected sTM tended to be lower before treatment ($86.8 \pm 23.8\%$) compared to the CGN group

Fig.4 Changes of corrected serum thrombomodulin (sTM). The data represent one sample or the average of two samples.

($98.7 \pm 18.3\%$), but the difference was not statistically significant (Fig.3). Levels of corrected sTM did not correlate with the numbers of affected organs (data not shown). In contrast, corrected sTM levels were significantly higher ($145.7 \pm 46.9\%$) in the early phase after beginning the treatment (within 30 days) compared to before treatment ($P < 0.001$), in remission ($104.7 \pm 38.6\%$; $P = 0.01$), and the CGN group ($P < 0.001$), respectively. Changes of corrected sTM in each patient are shown in Fig.4. Corrected sTM increased significantly in the early phase after beginning treatment and decreased during remission in each patient ($P = 0.008$ and $P = 0.03$, respectively).

Discussion

In this study, we aimed to determine whether blood TM levels could be a marker of vascular injury in patients with MPO-ANCA-associated vasculitis, in which the kidney is one of the main targeted organs. Although the blood levels of TM are regarded as an indicator of vascular endothelial disorders, blood TM levels are also known to be affected by renal dysfunction, because smaller fragmented blood TM is cleared by the kidneys.^{9,11,16} First, we found a strong positive correlation between sTM and sCr in patients with primary glomerulonephritis, in accordance with previous reports.^{9,11,16,23} In patients with renal dysfunction, blood TM levels have often been evaluated after correction for renal function.^{15,23-25} In this study, in order to eliminate the effect of renal function on sTM, we calculated the corrected TM using a regression line between sCr and TM in patients with CGN and evaluated the corrected sTM in the course of disease.

The major finding in this study was that serum levels of corrected sTM were not increased in patients with MPO-ANCA-associated vasculitis in the active

period before treatment. In cases of systemic lupus erythematosus, several reports demonstrated that elevated blood TM concentrations were detected in patients with active diseases, even after correction for renal function or the elimination of patients with deteriorated renal function.¹²⁻¹⁶ On the other hand, the results of blood TM in small-vessel vasculitis such as Wegener's granulomatosis and microscopic polyangiitis conflict when considering renal function. Hergelesell et al. reported that TM is higher in patients with small-vessel vasculitis (Wegener's granulomatosis and microscopic polyangiitis) than in patients with other diseases leading to decreased renal function.²³ Boomsma et al reported that sTM levels also rose before the moment of relapse and dropped below the baseline levels after treatment of patients with Wegener's granulomatosis. However, when corrected for renal function, these changes in sTM levels were no longer significant.²⁴ Our results also showed that sTM did not reflect disease activity when corrected for renal function.

The reason why corrected sTM did not increase during the active period of vasculitis is not clear. One possible explanation is that during vasculitis, the expression of TM is down regulated transcriptionally. Inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), were increased in the active stage of disease and were decreased in remission in patients with MPO-ANCA-associated glomerulonephritis.²⁶ TNF- α and IL-6 are known to suppress the TM expression of endothelium in vitro.^{27,28} We therefore supposed that TM on the endothelial cells of blood vessels is transcriptionally down regulated during the active period before treatment. Interestingly, levels of corrected sTM increased significantly within 1 month after treatment initiation, in contrast to the rapid decrease of CRP. In the early phase after treatment, transcriptional up-regulation of TM in the injured endothelium may occur under the condition of suppressed inflammatory cytokines. The mechanisms of opposite changes of corrected sTM and CRP before and after the initiation of treatment require further study.

In summary, sTM corrected by sCr was not increased in active MPO-ANCA-associated vasculitis before treatment. In contrast, sTM was significantly increased in the early phase after beginning treatment, and decreased in remission. Although blood TM is considered a useful marker of vascular injury in certain diseases, sTM does not correlate with disease activity in MPO-ANCA-associated vasculitis when corrected for renal function.

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