Evaluation of Plasma and Urinary Thrombomodulin, Interleukin-1β and Tumor Necrosis Factor-α Levels in Patients with Henoch-Schönlein Purpura

Takashi YAMAMOTO, Hiroshi MIYATA, Naohiko MORIGUCHI and Kazuo YOSHIOKA

Department of Pediatrics, Kinki University School of Medicine

Henoch-Schönlein 紫斑病における血漿と尿中の thrombomodulin, interleukin-1β, および tumor necrosis factor-α の変動についての検討

山本 隆, 宮田 嘉, 森口 直彦, 吉岡加寿夫

近畿大学医学部小児科

Abstract The plasma and urinary levels of thrombomodulin (TM), interleukin-1β (IL-1β), および tumor necrosis factor-α (TNF-α) were measured in 24 patients with Henoch-Schönlein purpura (H-SP); 11 patients without nephritis and 13 with nephritis. The plasma and urinary levels of TM increased during the acute phase of H-SP (≤2 weeks after disease onset). In patients without nephritis in the late phase (>2 weeks), the levels decreased significantly as compared to the levels in the acute phase. In patients with nephritis, whose hematuria measured 10/HPF or more, the plasma and urinary TM levels remained significantly elevated in the late phase. The plasma and urinary IL-1β levels did not show significant changes. The plasma TNF-α levels showed changes similar to those of TM, suggesting a specific relation between TM and TNF-α. From these results, it is suggested that systemic or renal vascular endothelial cell damage continues for more than two weeks after disease onset in patients with nephritis.

Key words : thrombomodulin, interleukin-1β, tumor necrosis factor-α, Henoch-Schönlein purpura

I. Introduction

Thrombomodulin (TM) exists as a cell surface glycoprotein that is widely distributed on vascular endothelial cells. It is known that TM plays a major role in the regulation of intravascular coagulation by inhibiting the procoagulant effect of thrombin and accelerating the anticoagulant effect of protein C. In recent years, it has been reported that TM was present in soluble form in circulating blood plasma and urine, but its functions have not yet been fully clarified. Moreover, tumor necrosis factor (TNF), endotoxin and interleukin-1 (IL-1) have been reported to cause endothelial cells...
to synthesize a protein trigger for clotting and suppress TM expression.\cite{6-8}

Since Henoch-Schönlein purpura (H-SP) is characterized by bleeding of the skin and gut, and is accompanied by renal disease, the mechanism whereby complement and immunoglobulin abnormalities are transformed into abnormalities of hemostasis is of considerable interest. In this study, we measured the TM, IL-1β and TNF-α levels of the plasma and urine from patients with H-SP to examine the mechanism of endothelial cell injuries and determine whether TM can serve as a useful clinical marker for the severity of this disease.

II. Subjects

The subjects were 24 patients with H-SP. They were diagnosed from clinical findings and treated at Kinki University School of Medicine. The patients were divided into two groups by the presence/absence of nephritis. The 11 patients without nephritis (five males and six females, 6.2±4.1 years old) had normal urinalysis. The 13 patients with nephritis (10 males and three females, 8.7±4.1 years old) were found to have persistent abnormalities of urinalysis, and were further divided into three classes by degree of hematuria (0 ≤ RBC < 10/HPF, 10/HPF ≤ RBC < 30/HPF, 30/HPF ≤ RBC). There was no patient whose serum creatinine value increased more than 1.0 mg/dl and fell into renal failure. Four of the patients were treated with prednisolone for acute abdominal pain. As a control group, 15 patients (10 males and five females, 8.5±5.2 years) consulting our hospital for short stature or headaches, and having no significant disorders, were studied. Informed consent was obtained from the parents of all patients.

III. Methods

Fresh morning urine was obtained from the patients and control subjects and centrifuged at 2,500 rpm for 10 minutes. A part of the urine supernatant was used for the measurement of creatinine by Creatinine-Test Wako (Wako Junyaku, Osaka, Japan). The remaining urine, after having been dialyzed against Dulbecco's phosphate buffered saline (Flow Lab., Oxford, UK) using a System 500 Microdialyzer (Pierce, Rockford, USA) at 4°C for 2 h, was frozen and stored at −60°C until the assay. Venous blood was collected into 3.5% sodium citrate (nine parts blood, one part anticoagulant) and heparin (10 U/ml) from each subject, and then centrifuged at 2,500 rpm for 10 minutes. The plasma samples were also frozen at −60°C until the assay.

The concentrations of TM in the urine and plasma (sodium citrate added) were measured by an enzyme-linked immunosorbent assay (ELISA) using TM-Test Teijin (Teijin Diag, Osaka, Japan).\cite{9}

The concentrations of IL-1β and TNF-α in the urine and plasma (heparinized) were measured using IL-1β EASIA and TNF-α EASIA kits (Medgenix, Fleurus, Belgium). In these assays, the minimal detectable levels were 2.5 TU/ml for TM, 10 pg/ml for IL-1β and 5 pg/ml for TNF-α. The urinary excretion rate is expressed as a value corrected with the urinary creatinine (Cr) concentration.

IV. Statistical Analysis

For statistical analysis, samples below the detection limits were assumed to be at half of their detectable values. Differences of data were analyzed by the Wilcoxon-Mann-Whitney test. p values of <0.05 were considered significant.

V. Results

1. TM levels in plasma and urine (Fig. 1)

The mean ± standard deviation (SD) of the plasma and urinary levels of TM in the control group were 10.5±3.3 TU/ml and 33.2±16.1 TU/mg Cr, respectively. In the H-SP patients, the mean values of plasma TM within two weeks after disease onset (acute phase) were significantly higher than those of the control group (p < 0.01). In the H-SP patients without nephritis, the elevated values decreased as the disease progressed more than two weeks after the onset of illness (late phase, 45±15 days after onset). The patients with nephritis had elevated plasma TM levels continuously even in the late phase (95±98 days after onset) (p <0.01). The mean value of urinary TM during the acute phase was higher than that of the control group, similar to the result for plasma TM. In the H-SP patients without nephritis, the elevated values decreased as the disease progressed more than two weeks after the onset of illness (late phase, 45±15 days after onset). The patients with nephritis had elevated plasma TM levels continuously even in the late phase (95±98 days after onset) (p <0.01).

The mean value of urinary TM during the acute phase was higher than that of the control group, similar to the result for plasma TM. In the H-SP patients without nephritis, the elevated values decreased as the disease progressed more than two weeks after the onset of illness (late phase, 45±15 days after onset). The patients with nephritis had elevated plasma TM levels continuously even in the late phase (95±98 days after onset) (p <0.01). There was a significant correlation between the plasma and urinary TM levels in the patients without nephritis (r=0.73, p <0.05). There was, however, no significant correlation in the patients with nephritis.

2. IL-1β levels in plasma and urine (Fig. 2)

The mean ± SD of the plasma and urinary levels of IL-1β in the control group were 12.4±3.5 pg/ml
and 53.4±34.2 pg/mg Cr, respectively. In the H-SP group, the mean values of plasma and urinary IL-1β were not significantly different from those of the control group, although some patients with nephritis showed remarkably elevated levels of plasma or urinary IL-1β in the late phase. There was no significant correlation between the plasma and urinary IL-1β levels.

3. TNF-α levels in plasma and urine (Fig. 3)

The mean ± SD of the plasma and urinary levels of TNF-α in the control group were 7.8±2.2 pg/ml and 11.3±12.6 pg/mg Cr, respectively. The mean values of plasma TNF-α during the acute phase were significantly higher in the patients with H-SP (p<0.05) than in the control group; the same result as for plasma TM. In the H-SP patients without nephritis, the elevated values decreased as the disease progressed in the late phase (p<0.05). The patients with nephritis had elevated plasma levels continuously in the late phase, and the levels were remarkably high in the patients whose hematuria was 10/HPF or more. A weak but significant correlation was found between TNF-α and TM levels in plasma (r=0.39, p<0.05). The mean value of urinary TNF-α in the patients with H-SP was not significantly different from that of the control group, although some patients with nephritis showed remarkably elevated levels in the late phase. There was no significant correlation between the plasma and urinary TNF-α levels.

VI. Discussion

In recent studies, it has been demonstrated that TM is present in soluble form in plasma and urine, and that the level of TM increases during various disease states.4,5,9–12) A common mechanism for increased plasma TM is presumed to reflect endothelial cell damage.13) In this study, we measured the concentration of TM from H-SP patients with and without nephritis, and found that the mean values of plasma and urinary TM during the acute phase were significantly higher than those of the
Thrombomodulin in Patients with Henoch-Schönlein Purpura

These elevated plasma TM levels are also presumed to reflect endothelial cell damage. In addition, even more than two weeks after the onset of illness, the plasma TM levels remained elevated in patients with nephritis. Although previous studies have shown that the renal clearance of TM may influence TM plasma levels, there was no patient in our study whose serum creatinine values increased more than 1.0 mg/dl and who fell into renal failure. Therefore, this result suggests that endothelial cell damage continued even then in these patients. The relationship between plasma and urinary TM levels in patients without nephritis, who did not reveal characteristic changes. However, the mean values of plasma TNF-α levels remained in the late phase. Hence, the plasma TNF-α levels had changes similar to those of the renal TM levels. Although IL-1β and TNF-α are pleiotropic mediators which share numerous inflammatory properties and contribute to acute and chronic inflammatory changes, plasma IL-1β levels were not related to changes in plasma TNF-α levels. TNF-α is related to the activation of coagulation and has been reported to modulate endothelial cell functions. There is a report that TNF-α acts competitively with TM product.

Cytokines exist widely in plasma and various body fluids. They play important roles as mediators of inflammation or the immune system, and as progressive factors in various disorders. In H-SP, the mean values of IL-1β in the plasma and urine did not reveal characteristic changes. However, the mean values of plasma TNF-α in the acute phase were significantly higher than those of the control group, and in the patients with nephritis whose hematuria measured 10/HPF or more, elevated values remained in the late phase. Hence, the plasma TNF-α levels had changes similar to those of the plasma TM levels. Although IL-1β and TNF-α are pleiotropic mediators which share numerous inflammatory properties and contribute to acute and chronic inflammatory changes, plasma IL-1β levels were not related to changes in plasma TNF-α levels. TNF-α is related to the activation of coagulation and has been reported to modulate endothelial cell functions. There is a report that TNF-α acts competitively with TM product.
In this study, plasma TNF-α levels were significantly related to plasma TM levels. Moreover, endotoxin and TNF-α were reported to be important stimulants for liberation of TM from endothelial cells. Thus, in spite of the down-regulation of TM expression secondary to inflammatory response, soluble TM in circulating blood may be increased by the cleavage of membrane-bound TM. In H-SP patients, some factors may influence endothelial cell damage, resulting in a change in the TM expression on the endothelial cell surface and an increase of the TM in circulating blood.

In summary, H-SP patients with nephritis had increased levels of plasma and urinary TM even in the late phase, suggesting the persistent presence of endothelial cell damage. It is proposed that the measurement of TM and TNF-α concentrations can be helpful to assess the state of endothelial cell damage.

This work was presented in the 37th Annual Meeting of the Japanese Society of Pediatric Hematology, Kofu, September 28-29, 1995.

References


