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CLINICAL STUDY

Soluble P-selectin glycoprotein ligand – a possible new target in ulcerative colitis

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Abstract: *Background:* The main characteristic of active inflammatory bowel disease (IBD) is the neutrophil infiltration into the intestinal lamina propria, where neutrophils usually do not reside. Selectins are cell surface glycoproteins responsible for binding the leukocytes to vascular cells and their extravasation into the surrounding tissue. They show high affinity to P-selectin glycoprotein ligand-1 (PSGL-1) receptors. PSGL-1 is expressed on the surface of all leukocytes and they mediate the rolling of neutrophils on P-selectin. Soluble PSGL-1 acts competitively with cellular PSGL in many physiological and pathological processes.
The aim of our study was to compare serum sPSGL-1 concentration in the blood of patients with ulcerative colitis (UC) and healthy control subjects.
Methods: Serum concentrations of sPSGL-1 were measured in 20 patients with UC and 20 control subjects. Two-layer immunoenzyme procedure (ELISA) was used.
Results: The mean (± standard deviation) serum concentrations of sPSGL-1 in patients with UC and controls were 349.97±75.40 U/mL and 284.39±52.40 U/mL, respectively (p=0.003).
Conclusion: In the present study, we showed that patients with UC had significantly higher sPSGL-1 blood values in comparison with healthy subjects. A short-term blockade with anti-PSGL-1 antibodies could block the transport of neutrophils and decrease UC activity. Thus it could possibly be employed in a new therapeutic approach to the treatment of UC *(Fig. 1, Ref. 25)*. Text in PDF *www.elis.sk.*
Key words: ulcerative colitis, P-selectin glycoprotein ligand-1.

Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) of unknown etiology; however, aberrant intestinal mucosal immune responses seem to play a role in the course of the disease (1). According to a hypothetical model of IBD pathogenesis in genetically predisposed individuals, some factors, both exogenous (infection, luminal bacteria) and endogenous (membranous role, blood supply, neurons) stimulate the mucosal immune response characterized by tissue inflammation and destruction (2–5).

In IBD, the expression of adhesion receptors on endothelial cells is increased, which facilitates the neutrophils to enter the intestinal lamina propria (6).

The main characteristic of active IBD is the neutrophil infiltration into intestinal lesions (7). Stimulated by chemotactic mediators (interleukin (IL)-8, leukotriene B4 and others), the neutrophils migrate from the blood into the intestinal lamina propria, where they usually do not reside. After entering the tissue, neutrophils become exposed to IL-1, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF) or lipopolysaccharides (LPS) and abundantly produce reactive oxygen species. Even the extracellular matrix itself may induce the activation of neutrophils, which then actively penetrate through the epithelium into and out of the intestinal lumen (6–16). Neutrophils are agents most responsible for tissue damage in IBD while their role, especially in UC, has not been sufficiently investigated (6, 16).

Selectin family, i.e. P-, E- and L-selectins, belong to cellular adhesion molecules. They are cell surface glycoproteins responsible for initial binding of leukocytes to the vascular endothelial cells and their consequent rolling along the endothelium and extravasation into the surrounding tissue (17).

L-selectin is expressed on leukocyte surfaces, whereas P- and E-selectins are expressed on activated endothelial cells. P-selectin is also expressed on activated platelets (18).

Selectins are involved in migration of neutrophils, monocytes, T lymphocytes, and platelets. Selectin or selectin ligand deficiency results in recurrent bacterial infections and chronic disease (19).

Selectins loosely bind to sialyl-Lewis X (sLeX)-similar glycans, but show high affinity to P-selectin glycoprotein ligand-1 (PSGL-1) receptors (17). PSGL-1 is a transmembrane receptor that forms homodimers via disulphide bonds. It consists of two identical glycoprotein chains of 120 kD.

PSGL-1 contains numerous sialylated, fucosylated O-linked oligosaccharide branches, many of which end in sLex epitope.

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In addition to fucose and sialic acid, PSGL-1 and P-selectin interaction requires at least one tyrosine-sulphate at the amino-terminal sequence. PSGL-1 is expressed on the surface of all leukocytes and they mediate the rolling of neutrophils on P-selectin. PSGL-1 serves as a ligand for L-selectin in neutrophil-neutrophil interactions (18, 20). In mice with deletion of the gene encoding PSG-1, the attraction of neutrophils to vascular wall is slowed down and moderate neutrophilia (triple increase) is present, which is similar to mice without the gene encoding P-selectin (19). Consequently, selectin or selectin ligand deficiency results in recurrent bacterial infections and chronic disease in both animals and humans.

There is a rare disease known as leukocyte adhesion deficiency type II (LAD-II). Individuals with this disease carry a mutation of the gene encoding the fucose transporter, leading to ineffective incorporation of fucose into PSGL-1. Consequently, neutrophils do not migrate to the infection site and bacterial inflammation thus remains constantly present (19).

Monoclonal Pt on PSGL-1 recognizes the epitope within the tyrosin-sulphate segment and blocks the recognition of P-selectin (21). Soluble PSGL-1 also has the ability to bind P-selectin and acts competitively with cellular PSGL in many physiological and pathological processes.

The inhibition of thrombocyte-leukocyte binding with recombinant SGL-1 Ig (ligand with high affinity to P-selectin) has shown beneficial effects in animal models of carotid angioplasty by reducing significantly the adhesion of neutrophils and platelets to the damaged arterial surfaces (22). Vowinkel et al (23) showed that the attraction of leukocytes and platelets to the inflamed colonic venules in mice with colitis induced by dextran sodium sulphate (DSS) were mutually associated and included in the interaction between P-selectin expressed on endothelial cells and platelets and PSGL-1 on the leukocyte and endothelial cell surfaces. The treatment with anti-PSGL-1 antibodies significantly reduced the inflammation in DSS-induced colitis (23).

The aim of our study was to compare serum sPSGL-1 concentration in the blood of patients with UC and healthy control subjects. The sPGL-1 concentration was expected to be higher in the blood of patients with UC.

Methods

The study was performed at the Split University Hospital Center from June to December 2012 and included patients with UC who underwent colonoscopy and had histological evidence of UC. Two patients were excluded due to corticosteroid and immunobiological forms of therapy. The study group included 20 patients (9 men and 11 women) aged between 19 and 65 (median, 42 years). Of them, 19 patients were taking aminosalicylic acid (ASA) preparations. The control group consisted of 20 healthy volunteers (18 men and 2 women) without the history of gastrointestinal diseases, who were recruited from blood donors aged between 21 and 64 years (median, 38.5 years).

Blood samples were collected from patients with UC and healthy controls and stored after centrifugation at −20 °C until analysis. The sandwich enzyme-linked immunosorbent assay (ELISA) was performed on a semiautomatic mini-Boss analyzer (Biomedica Medizinprodukte GmbH, Vienna, Austria) in order to quantitatively determine the serum concentration.

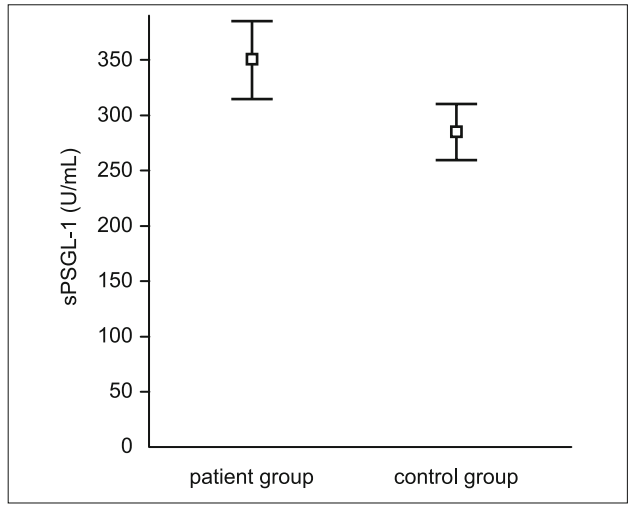

Fig. 1. Serum P-selectin glycoprotein ligand-1 (sPSGL-1) concentration in patients with ulcerative colitis (UC) and healthy controls. Squares – arithmetic mean; whiskers – standard deviation.

Serum sPSGL-1 concentration measured by ELISA was determined from the standard curve using standards with known concentration (from 1.6 to 50 U/ml). According to the manufacturer, the assay sensitivity is less than 1.0 U/ml. Human sPSGL-1 Platinum ELISA (eBioscience) tests were used, and serum samples were stored at −20 °C until analysis.

Statistical analysis

Statistically significant difference in sPSL-1 concentration between patients with UC and healthy controls was analyzed with Student t-test. The results were presented as mean ± standard deviation (SD). The level of significance was set at $p < 0.05$. All analyses were performed with Statistical Package for Social Sciences 17.0 (SPSS Inc., Chicago, IL, USA).

Results

The mean (± standard deviation) serum concentrations of sPSGL-1 in patients with UC and healthy controls were 349.97 ± 75.40 U/mL and 284.39 ± 52.40 U/mL, respectively ($p = 0.003$) (Fig. 1).

Discussion

Neutrophils are the most responsible for tissue damage in IBD. Neutrophil membranes contain selectins, while PSGL-1 binds to selectins located on neutrophil, thrombocyte, and endothelial cells, and partly on lymphocytes. Monocytes and endothelial cells also express PSGL. PSGL-1 molecules can also be found circulating freely in the blood, having the same biological role. As a result of these interactions, the flow of neutrophils through blood vessels is reduced, as well as their attraction to the vascular surface and

extravasation. The production of non-functional PSGL-1 molecule has been found in patients with a rare disease called leukocyte adhesion deficiency type II (LAD-II). These patients carry a mutation on gene encoding the fucose transporter, which leads to prolonged inflammation (a consequence of inability of leukocytes to arrive to the inflammation site). The beneficial effects of selectin inhibition have so far been investigated in organ transplantation studies, prevention of restenosis after angioplasty, and stent placement (19).

Brown (24) found that PSGL-1 deficiency in mice alleviated DSS-induced colitis and reduced the number of CD4+ T lymphocytes and production of Th1 and T17 citokines. The administration of anti-PSGL-1 antibodies in mice reduced the total number of macrophages, dendritic cells, and B lymphocytes in the lamina propria, and decreased the expression of MHC-II on dendritic cells and macrophages (25).

In the present study, we showed that patients with UC had significantly higher sPSGL-1 blood values in comparison with healthy subjects. A short-term blockade with anti- PSGL-1 antibodies could block the transport of neutrophils and decrease UC activity; however, further studies are needed to investigate the possibility of employing these results in a therapeutic approach to the treatment of UC.

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