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rPSGL-Ig

Treatment of Acute Myocardial Infarction
Thrombolytic
P-Selectin Inhibitor

EN: 273496

Abstract

The association of acute myocardial infarction with thrombus formation, frequently occurring subsequent to plaque rupture, has made early thrombolytic therapy the standard of care for patients. P-selectin-mediated platelet-leukocyte interactions have been reported in unstable angina, after myocardial ischemia and in coronary angioplasty, among other pathophysiological situations. Development of P-selectin antagonists as adjunctive therapies would be useful for enhancing the thrombolytic effect of currently available agents, without the risk of bleeding and thrombocytopenia. rPSGL-Ig is a P-selectin inhibitor which may have potential in the prevention of restenosis, enhancing the effect of thrombolytic therapy by preventing platelet-leukocyte interactions in the injured vessel wall.

Description and Production

P-Selectin Glycoprotein Ligand-1 (PSGL-1) is a mucin-like, homodimeric, disulfide-bonded glycoprotein (1). A recombinant, soluble and chimeric form of PSGL-1, rPSGL-Ig, has been developed as an antagonist to P-selectin to prevent recurrence of thrombotic events. It enhances thrombolysis without the risk of bleeding and decreases inflammation. It is produced in Chinese hamster ovary (CHO) cells engineered to coexpress the critical carbohydrate modifying enzymes fucosyltransferase VII and core2 GlcNAc transferase (2). It comprises the first 47 amino acids from the *N*-terminal end of the extracellular domain of mature PSGL-1 fused at the hinge region of human IgG_1. Two hinge proximal amino acids at positions 234 and 237 within the IgG Fc portion are mutated to alanine to reduce both complement activation and Fc receptor binding (3).

Introduction

Targeting of circulating leukocytes to specific areas of tissue injury or microbial invasion is critical for wound repair and host defense. P-selectin and PSGL-1 are two molecules essential for the precise development of these events. These receptors mediate the migration of certain leukocyte subclasses from the bloodstream to the site of damage. If this process occurs in an uncontrolled manner it may lead to the accumulation of leukocytes resulting in inflammatory tissue damage (4).

The selectin family of adhesion molecules mediates the initial attachment of leukocytes to endothelial cells (5). This initial attachment is followed by firm adhesion and diapedesis at the site of tissue injury and inflammation. P-selectin is one member of the selectin family that is constitutively expressed in platelet α-granules and in the Weibel-Palade bodies of endothelial cells (6, 7). It is mobilized to the cell surface after exposure of platelets or endothelial cells to activating agents or inflammatory cytokines. P-selectin is an extended protein with a membrane-distal C-type lectin domain, which binds to its high-affinity receptor, PSGL-1 or CD162, an extended mucin expressed on leukocytes. Interaction of P-selectin with PSGL-1 initiates tethering and rolling adhesion of leukocytes on inflamed endothelial cells or adherent activated platelets (Fig. 1), and enables platelets or platelet microparticles to form bridges between leukocytes (8, 9).

The events described have been reported to occur after coronary angioplasty in a proportion that depends on the degree of injury, and have been correlated with restenosis (10-12). The acute response to arterial injury induced by angioplasty involves the adhesion of platelets and leukocytes. Adherent platelets release the content of their granules and readily express P-selectin (13). Further activated platelets promote mural thrombus formation and vasoconstriction. Both processes can be upregulated by activated neutrophils. These reactions are also accompanied by monocyte/macrophage accumulation that augment the inflammatory reactions, leading to the progression of restenosis. This acute response, which involves thrombotic and inflammatory responses, is followed by chronic and adaptive modifications in the vascular structure. Neointimal hyperplasia, derived from medial cell proliferation and migration toward the intima with eventual reduction in vascular lumen or restenosis are likely to occur after repeated injury (14). In the long term, these changes are bound to induce alterations in the hemodynamic conditions.

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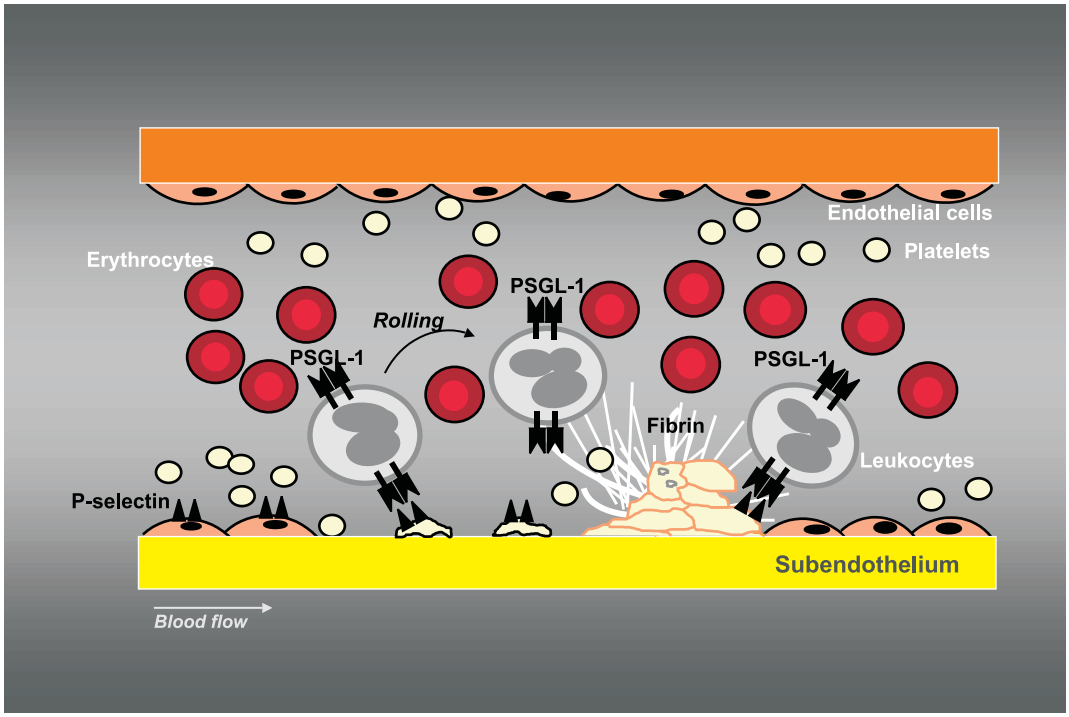

Fig. 1. The selectin family of adhesion molecules mediates the initial attachment of leukocytes to endothelial cells. P-selectin is mobilized to the cell surface after exposure of platelets or endothelial cells to activating agents or inflammatory cytokines. Interaction of P-selectin with PSGL-1, expressed on leukocytes, initiates tethering and rolling adhesion of leukocytes on inflamed endothelial cells or adherent activated platelets, and enables platelets or platelet microparticles to form bridges between leukocytes. Selectins contribute to both inflammation and thrombosis. Recombinant PSGL-Ig acts as an antagonist of these processes.

In addition, many models support a role for P-selectin in augmenting coagulation and thrombosis *in vivo*. Fibrin deposition in thrombi has been found to be P-selectin-dependent (15). A proposed hypothesis is that P-selectin promotes the recruitment of leukocytes or leukocyte microparticles that express tissue factor. These tissue factor-rich microparticles may accumulate on adherent activated platelets through P-selectin-PSGL-1 interactions in sufficient quantities to augment fibrin formation (16). A related hypothesis is that adhesion of monocytes and platelets via P-selectin may cause tissue factor release from monocytes (17), triggering coagulation and final fibrin formation. The interaction of platelets and neutrophils leads to the release of cathepsin G from neutrophils (18), which may result in endothelial damage with exposure of the subendothelial matrix and further activation of circulating platelets. Therefore, P-selectin antagonism either directly by specific antibodies or indirectly by blocking its counterreceptor in leukocytes, PSGL-1, may be a promising therapeutic strategy to prevent inflammation, thrombosis and procoagulant activity.

Pharmacological Actions

Inhibition of platelet/leukocyte binding with anti-P-selectin or the recombinant soluble form of PSGL-1 has been shown to accelerate pharmacological thrombolysis not only in primate and porcine models of arterial thrombosis but also in animal models of deep-vein thrombosis, myocardial and hepatic ischemia-reperfusion and intimal hyperplasia after angioplasty.

In an early study by Kumar *et al.* (19), rPSGL-Ig was administered in conjunction with the thrombolytic agent tissue plasminogen activator (tPA) in a porcine model of copper coil-induced thrombosis. An occlusive thrombus was formed in an internal iliac artery of Yorkshire pigs. The animals received heparin and, 15 min later, either vehicle or rPSGL-Ig (250 or 500 μg/kg) followed by infusion with 25 mg tPA. Lysis of the thrombus was significantly accelerated in pigs treated with rPSGL-Ig, and reocclusion was not observed in these animals. The results suggested that rPSGL-Ig enhances thrombolysis most likely by inhibiting the continuing adhesion of platelets to leukocytes, an interaction that leads to fibrin accretion. rPSGL-Ig appears to act locally at the thrombus site, since no differences in systemic coagulation/fibrinolytic parameters were detected.

Bienvenue *et al.* (20) demonstrated that administration of rPSGL-Ig inhibited platelet-leukocyte interactions at the sites of injury and reduced restenosis in a porcine model involving double arterial angioplasty. A further study by the same group (21) indicated that rPSGL-Ig inhibits circulating activated platelet binding to neutrophils induced by damaged arterial surfaces.

Using a porcine model of coronary artery balloon injury, Wang *et al.* (22) studied the effect of rPSGL-Ig on intimal hyperplasia. Balloon injury was induced in the left anterior descending and right coronary arteries of 16 pigs. Either 1 mg/ml of rPSGL-Ig or saline was administered 15 min before injury as an i.v. bolus. Results revealed an increased luminal area in the rPSGL-Ig-treated group. Additional immunohistochemistry and histological evaluation showed a significant decrease in the presence of tumor necrosis factor-α (TNFα), IL-1β and infiltration of macrophages in the injured vessel segments in the treated animals. It was concluded that rPSGL-Ig decreases neointimal hyperplasia following balloon injury by inhibiting the inflammatory and thrombotic responses at the site of balloon injury.

The effect of rPSGL-Ig on venous thrombosis has been investigated in baboons. Myers et al. demonstrated a dose-dependent inhibition of venous thrombosis and an increase in spontaneous recanalization at rPSGL-Ig concentrations ranging from 500 μg/kg to 4 mg/kg (23). In a more recent work (24), the same group investigated the effect of rPSGL-Ig on established venous thrombosis (VT) in baboons. The animals underwent 6 h of iliofemoral venous stasis to produce an occlusive VT and, 2 days later, they were treated for 14 days with 4 mg/kg of rPSGL-Ig, LMWH or saline. Treatment continued weekly (rPSGL-Ig) or daily (LMWH, saline). Findings from this study indicated that rPSGL-Ig is as effective as LMWH in treating established VT without anticoagulation, thrombocytopenia or wound complications.

The mechanisms by which rPSGL-Ig reduces thrombosis remain to be determined. One possible explanation is that rPSGL-Ig reduces adhesion of leukocytes or leukocyte microparticles at the sites of injury, attenuating the recruitment of procoagulant and proinflammatory mediators that increase thrombosis and tissue injury (9). Thus, P-selectin antagonism could favor the action of fibrinolytic agents via this mechanism.

Pharmacokinetics

The pharmacokinetics of rPSGL-Ig were determined using mice, rats, monkeys and pigs (25). The parameters estimated included C_{max} (maximum concentration), CL (clearance), V_c (volume of distribution), V_{ss} (volume of distribution at steady state), V_z (volume of distribution associated with terminal phase) and $t_{1/2}$ (terminal phase half-life). Allometric and pharmacokinetic/pharmacodynamic modeling was used to select doses for human clinical trials. According to Khor *et al.* (25), pharmacokinetic parameters of rPSGL-Ig such as CL, V_c and $t_{1/2}$ across animal species are well described by power functions with body weight (W) as an independent variable. The power functions for CL, V_c and $t_{1/2}$ are as follows: $CL = 0.37\ W^{0.93}$ ml/h [$r^2 = 0.94$); $V_c = 45.0\ W^{1.064}$ ml [$r^2 = 0.988$); $t_{1/2} = 190\ W^{0.159}$ h [$r^2 = 0.75$) and allow the prediction of rPSGL-Ig pharmacokinetics in humans. The doses that may provide potential effects in humans range from 0.13-4.7 mg/kg, which produce concentrations above those associated with efficacy in animal disease models and maintain concentrations above the EC_{50} of *in vitro* binding between rPSGL-Ig and stimulated human platelets.

The pharmacokinetics and tolerability of rPSGL-Ig have been assessed in healthy young and elderly volunteers in a double-blind, randomized, placebo-controlled trial that examined single ascending i.v. doses ranging from 0.035-7.0 mg/kg. The compound was well tolerated and no adverse effects were observed on bleeding time, platelet aggregation or the immune system. The disposition of rPSGL-Ig was multicompartmental, with an initial disposition phase of 1-2 weeks and a $t_{1/2}$ of 12.5-21.9 days. Mean C_{max} and AUC values increased with increasing dose, although the elderly showed reduced drug exposure. Mean CL and V_{ss} were 10 ml/h and 4 l, respectively. It was concluded that rPSGL-Ig disposition in humans is multicompartmental, with a low volume of distribution and a long elimination half-life. Dose proportionality was suggested across cohorts (26).

Clinical Studies

A multicenter, randomized, double-blind, placebo-controlled, dose ranging, safety and efficacy trial of rPSGL-Ig for use with thrombolytic therapy in patients with acute myocardial infarction (RAPSODY) has just recently been completed. During the preparation of this monograph, Wyeth Pharmaceuticals, who sponsored the trial, informed Neose Technologies of its intention to discontinue development of rPSGL-Ig for myocardial infarction due to disappointing results. Development for other indications may continue (27).

Source

Developed by Genetics Institute (Wyeth Pharmaceuticals). Neose Technologies, Inc. entered into a research, development and license agreement with Wyeth Pharmaceuticals in 2001 to apply GlycoAdvance™, a protein glycosylation technology, in the production of rPSGL-Ig.

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