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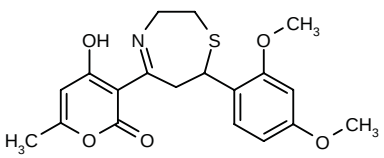
KF38789

Treatment of Ischemia Reperfusion Injury
P-Selectin Inhibitor

3-[7-(2,4-Dimethoxyphenyl)-2,3,6,7-tetrahydro-1,4-thiazepin-5-yl]-4-hydroxy-6-methyl-2*H*-pyran-2-one



$C_{19}H_{21}NO_5S$
Mol wt: 375.4387
CAS: 257292-29-8
EN: 297683

Abstract

P-selectin is a cell adhesion molecule of the selectin family and is mainly expressed on the cell surface of platelets and endothelial cells. P-selectin binds to the P-selectin glycoprotein ligand-1 (PSGL-1), which has sialyl Lewis^x residue on the extracellular domain. Inhibition of P-selectin ameliorates various diseases in animals such as stroke, ischemia reperfusion injury, lung injury and arthritis. KF38789 is a novel, low-molecular-weight inhibitor of P-selectin-dependent cell adhesion in physiological conditions. In the cell adhesion assay, KF38789 inhibited adhesion of the human leukemia cell line U937 to immobilized P-selectin immunoglobulin G chimeric protein (P-selectin-Ig) but had no effect on cell adhesion to E-selectin-Ig and L-selectin-Ig. P-selectin binding to sialyl Lewis^x was not inhibited by KF38789, and the compound reduced P-selectin-induced superoxide production from human polymorphonuclear cells. Thioglycollate-induced accumulation of leukocytes in mouse peritoneal cavity was also inhibited by KF38789. The development of small-molecule inhibitors, such as KF38789, that block the interaction of P-selectin with its natural ligands on the cell surface is an attractive therapeutic approach.

Synthesis

KF38789 was purchased from ChemBridge Corporation (code number 118638, San Diego, CA, USA).

Description

The analytical data for KF38789 by NMR and fast atom bombardment (FAB) MS are as follows. ^{1}H and ^{13}C NMR spectra were recorded at 30 °C on JEOL JNM-LA300 spectrometer by 300 MHz and 75 MHz, respectively. Chemical shifts were referenced to tetramethylsilane set at 0 ppm for proton and carbon. KF38789 was dissolved in chloroform-d ($CDCl_3$), and then TMS was added to the solution. FAB mass spectra were taken on a JEOL JMS-SX102AQQ spectrometer. Glycerol was used as a matrix. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.10 (d, J=0.8 Hz, 3H), 2.78 (ddd, J=2.5, 6.0, 14.8 Hz, 1H), 3.00 (ddd, J=2.9, 9.5, 14.9 Hz, 1H), 3.53 (dd, J=9.5, 13.4 Hz, 1H), 3.79 (s, 3H), 3.83 (s, 3H), 3.98 (m, 1H), 4.18 (m, 1H), 4.48 (dd, J=1.8, 9.5 Hz, 1H), 4.79 (broad d, J=13.4 Hz, 1H), 5.69 (d, J=0.8 Hz, 1H), 6.45 (d, J=2.6 Hz, 1H), 6.48 (dd, J=2.6, 8.2 Hz, 1H), 7.23 (d, J=8.2 Hz, 1H), ca. 14.3 (broad s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.8, 30.5, 35.3, 40.2, 46.8, 55.4, 55.8, 96.5, 99.1, 104.8, 107.5, 122.6, 128.1, 157.3, 160.4, ca. 163, ca. 163, 179.1, ca. 185. FABMS m/z 376 $[M+H]^+$. High resolution FABMS m/z 376.1206 $[M+H]^+$, calculated for $C_{19}H_{22}NO_5S$ 376.1219.

Introduction

Adhesion molecules contribute to the cell-cell interaction of leukocytes and vascular endothelial cells. These adhesion molecules are mainly classified into 3 groups, the integrin family, the immunoglobulin superfamily and the selectin family. These molecules play important roles in the extravasation of leukocytes into the tissue during an inflammatory response. The selectin family consists of 3 closely related cell surface molecules: L-selectin (CD62L), E-selectin (CD62E) and P-selectin (CD62P). Each of the selectins has a unique and characteristic

Ichiro Miki[1,*], So Ohta[2], Yasuko Inujima[1], Masayuki Abe[1], Youichi Uosaki[1], Soichiro Saro[1]. [1]Biomedical Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd., Shimotogari 1188, Nagaizumi, Sunto, Shizuoka 411-8731, Japan; [2]Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd., Asahi-machi 3-6-6, Machida, Tokyo 194-8533, Japan. *Correspondence.

extracellular region composed of an amino terminal calcium-dependent C-type lectin domain, an epidermal growth factor (EGF)-like domain and 2-9 short consensus repeat units homologous to domains found in complement binding proteins (1, 2). All 3 selectins bind to the carbohydrate ligand sialyl Lewis^x (sLe^x). P-selectin was originally discovered as the cell surface protein associated with platelet activation (3, 4). This molecule, which is stored in the α-granules in resting platelets, is rapidly redistributed to the cell surface upon activation. P-selectin is also found in the storage granules of endothelial cells, known as Weibel-Palade bodies (5). P-selectin in platelets and endothelial cells is rapidly moved to the cell surface by stimulation with thrombin, histamine or cytokines and mediates the cell-cell interaction of leukocytes, endothelial cells and platelets (3, 5). The P-selectin glycoprotein ligand-1 (PSGL-1), which has sLe^x residue on the extracellular domain, is known to be the specific ligand for P-selectin (6). Therefore, P-selectin is believed to be involved in a wide variety of inflammatory diseases (4, 6). Recently, anti-P-selectin antibodies were reported to inhibit stroke (7), ischemia reperfusion injury (8), cobra venom factor-induced acute lung injury (9) and arthritis (10). Physiological functions of P-selectin were also clarified in animal models. Thioglycollate-induced infiltration of neutrophils into the peritoneal cavity was decreased in P-selectin knockout mice (11). An expression of P-selectin *in situ* has also been observed in human inflamed tissue and platelets. P-selectin is coexpressed with intercellular adhesion molecule-1 (ICAM-1) in atherosclerotic endothelium on the luminal surface of the endothelial cells (12). Other studies showed that ICAM-1, vascular cell adhesion molecule-1 and P-selectin were expressed in the lung vascular endothelium of asthmatics (13). These data suggest that P-selectin plays an important role in human diseases especially in recruitment of inflammatory cells and platelets.

P-selectin also acts as a signaling molecule. P-selectin and platelet-activating factor synergistically enhanced the secretion of monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNF-α) by monocytes (14). Moreover, cross-linking of a ligand of P-selectin on mouse neutrophils induced the production of reactive oxygen intermediates (15). An engagement of PSGL-1 enhanced the tyrosine phosphorylation and activated mitogen-activated protein kinases in human neutrophils (16). These data indicate that P-selectin ligand is able to transduce an “outside-in” signal when engaged by P-selectin. The juxta-membrane region of PSGL-1 cytoplasmic tail was reported to bind moesin, which belongs to the band-4.1 superfamily of membrane-cytoskeleton-linking proteins (17). Leukocyte rolling, which was transfected with PSGL-1 without the cytoplasmic domain, was almost completely absent under the flow condition (18). Thus, attachment of PSGL-1 to the leukocyte cortical cytoskeleton is essential for leukocyte rolling on P-selectin. These results underline the role of moesin in the subcellular redistribution and activation of PSGL-1.

Anti-P-selectin antibodies and P-selectin gene knockout mice were usually used to evaluate the physiological roles of P-selectin. Several inhibitors of P-selectin have previously been reported. However, almost all of these inhibitors were either carbohydrates or their mimetics designed from sLe^x, one of the carbohydrate ligands of P-selectin (19-21). There are few reports of P-selectin specific low-molecular-weight inhibitors. We reported a novel low-molecular-weight inhibitor of P-selectin, KF38789 (22). A small-molecule P-selectin inhibitor, which has a molecular weight of 681 and quaternary ammonium salt with an IC_{50} value of 0.2 μM, was recently reported by Wyeth Research (23).

Pharmacological Actions

In vitro *activity*

P-selectin-dependent cell adhesion was measured using the human leukemia cell line U937 and P-selectin-Ig coated plate at room temperature for 30 min (22). KF38789 displayed a concentration-dependent inhibition of cell adhesion to immobilized P-selectin-Ig with an IC_{50} value of 1.97 μM (Fig. 1).

An anti-mouse P-selectin monoclonal antibody (MAb) RB40.34 also inhibited cell adhesion with an IC_{50} value of 0.41 μg/ml in this assay. KF38789 did not have any effect on E-selectin-Ig mediated adhesion of U937 at 100 μM or L-selectin-Ig mediated adhesion of HL60 at 100 μM (Fig. 1). Although integrin-dependent binding is known to decrease at low temperature, selectin-dependent

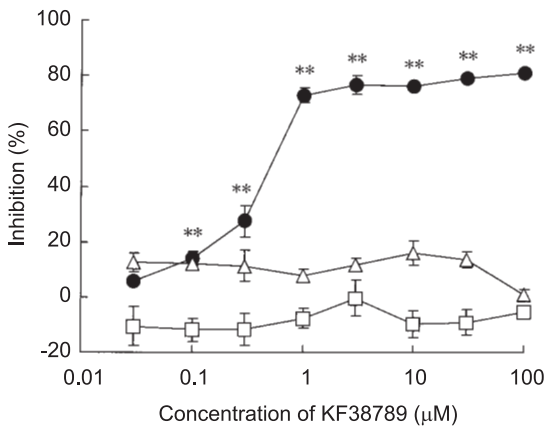


Fig. 1. Effects of KF38789 on the adhesion of cells to selectin-Igs. The adhesion of cells to immobilized P-selectin-Ig (●), E-selectin-Ig (□) and L-selectin-Ig (Δ) were measured in the presence of various concentrations of KF38789. U937 cells were used for the adhesion of P-selectin and E-selectin and HL60 cells were used for the adhesion of L-selectin. Data are presented as mean ± SEM of 3 experiments. *$p < 0.05$; **$p < 0.01$ compared with adhesion without drug (Dunnett test).

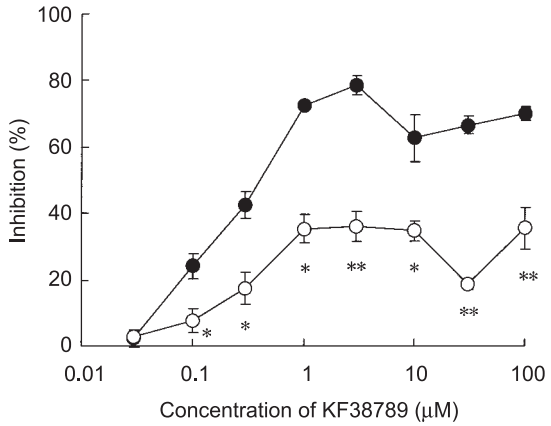


Fig. 2. Temperature-dependent inhibition of KF38789 on the adhesion of U937 to immobilized P-selectin-Ig. Binding assay of U937 cells to immobilized P-selectin-Ig was performed at room temperature (●) and 4 ºC (○) with various concentrations of KF38789. Data are presented as mean ± SEM of 3 experiments. *$p < 0.05$, **$p < 0.01$ compared with data at room temperature (Student's *t* test).

adhesion does not change at 4 °C. To clarify the temperature dependency, the effect of KF38789 on the adhesion of U937 to P-selectin-Ig was tested at 4 °C. Adhesion of U937 to immobilized P-selectin was obtained; however, the inhibitory effect of KF38789 was markedly decreased at 4 °C (Fig. 2). We tested the activity of KF38789 on the interaction of P-selectin with its ligand sLe^x to elucidate the compound's mechanism of action. Binding of P-selectin-Ig to sLe^x glycolipid-coated plate was measured in the presence of the compound. KF38789 did not show any inhibitory effect on P-selectin binding to sLe^x at a concentration of 100 μM (0.3% inhibition) (22). In this system, an anti-P-selectin MAb inhibited P-selectin binding with an IC_{50} value of 2.0 μg/ml. The IC_{50} value of the antibody correlated with that of U937/P-selectin-Ig binding. The small-molecule inhibitor reported by Wyeth Research inhibited PSGL-1 to immobilized soluble form of P-selectin on the plate (23). Although both KF38789 and the Wyeth compound specifically inhibited P-selectin-dependent adhesion, the two compounds exhibited different mechanisms on the adhesion of P-selectin.

KF38789 is a potent inhibitor of P-selectin-mediated cell adhesion at physiological conditions. The inhibition of KF38789 on P-selectin showed temperature dependency. Interaction of P-selectin and sLe^x was not interrupted by KF38789. Recently, the cytoplasmic domain of PSGL-1 was reported to bind with a cytoskeleton-linking protein (17) and regulate the avidity to P-selectin (18). These data indicate that KF38789 is likely to interact with the associated protein on P-selection or directly regulate the avidity of PSGL-1.

KF38789 regulated the P-selectin-dependent activation of leukocytes. P-selectin-Ig induced the release of superoxide from human polymorphonuclear leukocytes (PMN). KF38789 inhibited the superoxide production by PMN at concentrations of 0.1-10 μM in a dose-dependent manner (22). KF38789 did not inhibit phorbol myristate acetate (PMA)-induced superoxide production at 100 μM. Therefore, the inhibition of superoxide production by KF38789 appears to be dependent on the interaction of PMN with P-selectin.

In vivo *activity*

Peritoneal injection of thioglycollate induced the accumulation of leukocytes into the peritoneal cavity. BALB/c mice were treated with an intraperitoneal injection of 1 ml of 3% thioglycollate medium I. The number of leukocytes in the peritoneal cavity was increased about 800-fold at 6 h compared with that before injection. Intravenous administration of 1 mg/kg of KF38789 at 0 and 3 h significantly reduced neutrophil accumulation with 34% inhibition (22). An anti-P-selectin MAb, intravenously injected at 1 mg/kg, resulted in a 37% reduction in this assay. The efficacy of KF38789 was almost equal to that of an anti-P-selectin antibody.

Toxicology

Cell toxicity of KF38789 was assessed by cell viability using Cell Proliferation Kit II (XTT; Boehringer Mannheim). No effect on viability of U937 cells was shown by KF38789 at 100 μM during a 30-min incubation period. *In vivo* toxicity of KF38789 was monitored in mice for 6 h. KF38789 was injected at 1 mg/kg i.v. at 0 h and 3 h. No acute toxicity was observed under these conditions.

Clinical Studies

KF38789 is currently in preclinical studies.

Source

Kyowa Hakko Kogyo Co., Ltd. (JP); supplied by ChemBridge Corporation (US).

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