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The Role of MCP-1(Monocyte Chemoattractant Protein-1) in Restenosis

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Monocyte chemoattractant protein-1 (MCP-1) is a monomeric polypeptide with a low molecular weight that its major activity is associated with monocytes. The polypeptide causes the expression of e-selectin and the infiltration of monocytes into the subendothelial space by inducing a lasting impact on monocytes. MCP-1 activity also activates monocytes and the express of tissue factor (TF), superoxide anions and proinflammatory genes. Other roles identified for MCP-1 is the direct impact of polypeptide on restenosis after surgery. This review article is aimed to use the available resources of database in relation to MCP-1 to discuss in detail about the clinical significances, the signaling pathways in which MCP-1 play a role, gene polymorphism and its effects, the role of MCP-1 in vascular disease, particularly the role in the artery reclogging and finally the gene therapy mediated by MCP-1- inhibition on restenosis.

Key words: MCP-1, Restenosis, Monocytes.

Restenosis is a narrowing of a blood vessel that cause blood flow restriction. Restenosis usually appertain to an artery or other large blood vessel that has become narrow. The procedures that frequently used to treat the vascular damage from restenosis of blood vessels include vascular surgery, cardiac surgery, and angioplasty. Devices (e.g., stent-grafts, balloon angioplasty, etc.) and location of procedure make difference rates of restenosis. In cardiac procedures, balloon angioplasty has been associated with a high incidence of restenosis, with rates ranging from 25% to 50%, and the majority of these patients need further angioplasty within 6 months[1]. In peripheral procedures, rates are still high. A 2003 study of selective and systematic stenting for limb-threatening ischemia reported restenosis rates at 1 year follow-up in 32.3% of selective stenting patients and 34.7% of systematic stenting patients[2].

Damage to the blood vessel wall by angioplasty triggers physiological response that can be divided into two stages. The first stage that occurs immediately after tissue trauma, is thrombosis. A blood clot forms at the site of damage and further hinders blood flow. This is accompanied by an inflammatory immune response.

The second stage tends to occur 3–6 months after surgery and is the result of proliferation of cells in the media, a smooth muscle wall in the vessel. This is also known as Neointimal Hyperplasia (NIHA)[3].

Monocyte chemoattractant protein-1 (MCP-1), is a monomeric polypeptide with 9,000 to 15,000 Da molecular weight. It's the C-C chemokine

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_ subfamily prototype and its most potent activity is toward monocytes. It rapidly causes a change on monocytes to adhere firmly onto endothelial cells expressing E-selectin[4] and monocyte infiltration into the subendothelial space[5]. besides monocyte functions, MCP-1 activates monocytes and causes the expression of tissue factor (TF), superoxide anions, and proinflammatory genes[6].

The arterial wall cells, including endothelial cells, smooth muscle cells (SMC), and macrophages, produce Monocyte chemoattractant protein-1 in response to various stimuli. Cytokines[7], minimally modified low-density lipoprotein (MM-LDL)[8], angiotensin II[9], homocysteine[10], shear stress[11], and activated platelets[12] induce MCP-1 expression in these cells.

It showed that MCP-1 identical to *JE*, a gene that platelet-derived growth factor persuade its expression in mouse fibroblasts[13]. However, its human homolog, CCL2, was purified from human cell lines on the basis of its monocyte chemoattractant properties.

The first discovered human CC chemokine was CCL2. Its gene location is on chromosome 17 (chr.17, *q11.2*), human MCP-1 is contain 76 amino acids and is 13 kDa [14]. Different molecular mass forms of CCL2 have been discovered, and it seems to be caused by O-glycosylation. Glycosylation of CCL2 reduce its chemotactic potency.

Mutational analysis of CCL2 has showed two critical region for biological activity in its primary structure[15]. One of these regions consists of the sequence from Thr-10 to Tyr-13, and the other one consists of residues 34 and 35. CCL2 activity decreasing was showed in mutation of either residue 10 or 13 [16]. Two type of mutation was studied in the second region, one of them by insertion of a proline between Ser-34 and Lys-35, and the other one by a replacement of those two residues with the Gly-Pro-His sequence. These two mutations was showed same results, CCL-2 activity reduction. In addition, mutation of residues 28 and 30 are impressive on cell-type specificity[15]. Further, deletion of residues at the N-terminal causes of a loss of CCL2 activity[17] although it showed that some of these N-terminus deletion mutants cause CCL2 antagonists[18].

Clinical importance of MCP-1

CCL2 is involved in pathogeneses of several diseases characterized by monocytic infiltrates, such as psoriasis, rheumatoid arthritis and atherosclerosis[19]. Administration of anti-CCL2 antibodies in a model of glomerulonephritis reduces infiltration of macrophages and T cells, reduces crescent formation, as well as scarring and renal impairment[20].

Hypomethylation of CpG sites of the CCL2 promoter region is affected by high levels of blood glucose and TG, which increase CCL2 levels in the blood serum. The later plays an important role in the vascular complications of type 2 diabetes[21].

Molecular biology of MCP-1

CCL2 is a monomeric polypeptide, with a molecular weight of approximately 13 kDa. CCL2 is anchored in the plasma membrane of endothelial cells by glycosaminoglycan side chains of proteoglycans. CCL2 is primarily secreted by monocytes, macrophages and dendritic cells. Platelet derived growth factor is a major inducer of CCL2 gene. To become activated CCL2 protein has to be cleaved by metalloproteinase MMP-12.CCR2 and CCR4 are two cell surface receptors that bind CCL2[22].

CCL2 exhibits a chemotactic activity for monocytes and basophils. However, it does not attract neutrophils or eosinophils. After deletion of the N-terminal residue, CCL2 loses its attractivity for basophils and becomes a chemoattractant of eosinophils. Basophils and mast cells that are treated with CCL2 releases their granules to the intercellular space. This effect can be also potentiated by a pre-treatment with IL-3 or even by other cytokines. CCL2 augments monocyte anti-tumor activity and it is essential for formation of granulomas[23, 24].

Cell signaling and MCP-1

CCL2 can be found at the sites of tooth eruption and bone degradation. In the bone, CCL2 is expressed by mature osteoclasts and osteoblasts and it is under control of nuclear factor ºB (NFºB). In the human osteoclasts, CCL2 and RANTES (regulated on activation normal T cell expressed and secreted). Both MCP-1 and RANTES induce formation of TRAP-positive, multinuclear cells from M-CSF-treated monocytes in the absence of RANKL, but produced osteoclasts that lacked cathepsin K expression and resorptive capacity. It is proposed that CCL2 and RANTES act as

autocrine loop in human osteoclast differentiation[25].

The CCL2 chemokine is also expressed by neurons, astrocytes and microglia. The expression of CCL2 in neurons is mainly found in the cerebral cortex, globus pallidus, hippocampus, paraventricular and supraoptic hypothalamic nuclei, lateral hypothalamus, substantia nigra, facial nuclei, motor and spinal trigeminal nuclei, gigantocellular reticular nucleus and in Purkinje cells in the cerebellum[26].

Cambien and colleagues used Mono-Mac6 cell line to examine the mcp-1 intermediate receptor in the signaling pathway of the trans-endothelial migration of monocytes. The stimulation of mcp-1, with a specific period of time, is influenced by the extracellular signals of erk1 and erk2 kinases and also depends on sapk1/jnk1 and sapk2/p38 protein kinases[27]. Sapk1/jnk1 was blocked by piceatannol that shows its regulation by syk kinase, while sapk2/p38 activation is suppressed by pp2 that obviously shows the upregulation by src kinase. The erk activation was not influenced by pp2 and piceatannol. Pertussis toxin, a blocker of Go/Gi protein, prevents the activation of mcp-1 affected by erk, with no impact on the sapk1/jnk and sapk2/p38 activity. The results indicate the influence of Go/Gi protein and nonreceptor tyrosin kinase in the early MCP-1 signaling. Moreover, chemotaxis and the MCP-1 dependent migration of trans-endothelial were decreased significantly by a high concentration of SB202190, the general inhibitor of SAPK or by SB203580, the specific inhibitor of spak2/p38, and were completely ceased by pertussis toxin. Overall, the results showed that the coordination of distinct signaling pathways is needed to create a perfect response to mcp-1 for transmission of monocyte[28]

MONZA ME and colleagues investigated how mcp-1 and ccr2b receptor complex impacts smokers. Cigarette smoke is a risk factor in the development of chronic obstructive pulmonary disease (copd), respiratory disease of airflow obstruction, mucus hypersecretion, chronic inflammation, and upregulation of inflammatory mediators such as mcp-1[29-32]. Mcp-1 causes chemotoxin impacts and activates [44/42]MAPK, an effective Kinase in the regulation of mucin in bronchial epithelium. During the study, they used normal human bronchial epithelial cells (NHBE). They showed that nhbe cells release the mcp-1 to the epithelial surface and cause the expression of ccr2b receptor isoform in apical pole. In addition, they introduced a new activity for the mcp-1 which increases two major respiratory track mucins MUC5AC and MUC5B, and finally activates the cascade pathways mediated by the interaction of mcp-1 and Ccr2b receptors with Gq subunit, followed by activating PLC², PKC, and MAPK. It also showed that mcp-1 is capable of self-regulation and increasing its expression through a different pathway, in which RhoA GTPase is involved. They also stated that just a single exposure to mcp-1 is sufficient to induce the Mcp-1 secretion and the stability of mucin upregulation was shown till 7 days after initial exposure[33]. (figure1)

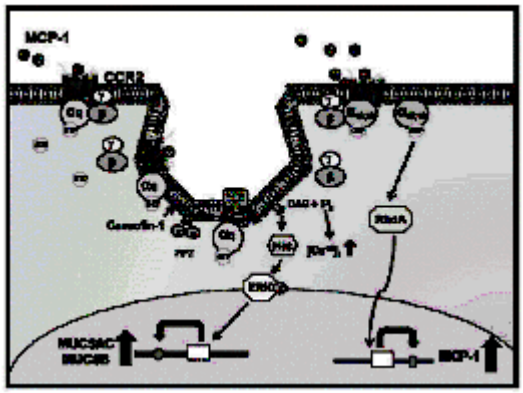

Fig. 1. Schematic view of ccr2b signal transduction, indicating the expression of mucin and the positive self- regulation of mcp-1 [33]

M. Carmen and colleagues also found that activation of erk by mcp-1 is mediated by th einvolvement of heterotrimeric Gi, protein kinase c, phospho-inositid-3-kinase and Ras subunits. It was also showed that the activity of cytosolic tyrosine kinase, the transactivation of epidermal growth factor receptor and the changes in calcium level are not required for the mitogenic activity caused by mcp-1. They also found that the internalization of ccr2b is not essential alone to activate erk by mcp-1, although dynamin mutant partially inhibits erk. The overall results showed that the parallel pathways are activated for the complete activation of mitogen-dependent kinase cascade and the internalization of other signaling

proteins. However, the receptor is not only required for the complete activation of erk of not only[34]. Transmission of monocytes into subendothelial space is an early step in the formation of plaque and inflammation. The activity of chemotoxin and integrin are an important function in the transmission of monocytes[35, 36]. N. Ashida and colleagues investigated the signaling cascade of integrin activation and chemotaxis by MCP-1, the role of MAPK and Rho GTPAse in THP-1 cells, monocyte cell lines. Mcp-1 stimulates the β1 integrin dependent cell adhesion. The MCP-1 mediated cell adhesion is inhibited through MEK inhibitor, but not the p38-MAPK inhibitor. In contrast, mcp-1 dependent chemotaxis is inhibited by p38-mapk inhibitor, but not influenced by mek inhibitors. mcp-1 dependent chemotaxis is also inhibited by Rho GTPase, C3 exoenzyme and Rho kinase but not influence on the integrin-dependent cell adhesion. Moreover, the c3 exoenzyme and the Rho kinase inhibitor inhibit the p38-MAPK activation of MCP-1 dependent pathway. Their data showed that ERK is responsible for integrin activation, p-38-MAPK and Rho are responsible for chemotaxis mediated by mcp-1 and also Rho and Rho kinases are located in the mcp-1 dependent signaling pathway in the upstream of p38-mapk. Generally and finally, the results showed that two distinct MAPK controls two distinct pathways of mcp-1 dependent activation of integrin and chemotoxis in thp-1 [37]. (figure2)

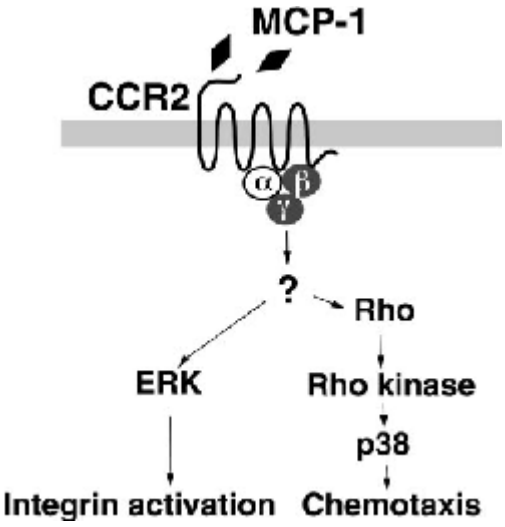

Fig. 2. MCP-1-mediated cascade for chemotaxisand integrin activation

MCP-1 gene polymorphism

Regarding the possibility of the effect of genetic polymorphisms in the regulatory region of mcp-1, which increases the expression of chemokine[38], C. czali and colleagues in 2001examined the role of this polymorphism detection simultanously with SDF-1_810A chemokine and chemokine receptor mutant CCR2-CCR5(delta-mosalase bozorg) 32 and 64I in 318 patients with coronary artery disease (CAD) for coronary artery bypass surgery compared with 320 healthy subjects. Their results showed that the level of homozygous MCP-1_2518 G/G was significantly higher in CAD patients than controls. The levels of Lp (a) in CAD patients with G/G genotype was significantly higher than patients with G/A and A/A. No homozygous patient was found for the chemokine mutant CCR2-CCR5 (delta-mosalase bozorg) 32 and 64I . Studies showed that the genotype distribution of two alleles in patients had a deviation from Hardy-Weinberg equilibrium, indicating the significant low number of homozygous than expected. It seems that Mcp-1_2518G variant in the homozygous state is an important and effective genetic factor in CAD . This genotype is associated in the patients with the level of Lp (a). Also, homozygous individuals for the chemokine mutation CCR2-CCR5 (delta-mosalase bozorg) 32 and 64I are at lower risk of CAD[39].

Role of MCP-1 in vascular injury

Taubman et al reported MCP-1 mRNA presence following balloon injury at 4 h in a rat carotid mode[40]. The inhibitory effect of polyclonal IgG of goat against MCP-1 in a rat carotid injury model had been reported by Furukawa et al[41]. Also, Gu et aldemonstrated consequential reductions in lipid and monocyte agglomeration in the aortas of mice because of genetically lacking in MCP-1 and the LDL receptor. Mice with deficient in CCR2 and apoE showe less extensive atherosclerotic lesion development[42].After that, Ni et al have reported effectiveness of anti-MCP-1 gene therapy against atherosclerotic lesion development in an apoE knockout mouse sample[43]. The findings of Frederick GP Welta et al study confirm the earlier findings of Taubman et al, study that shown peak levels of MCP-1 mRNA within hours after balloon injury. Also they showed that MCP-1 mRNA is stable as long as 14 days post stent implantation[44].

Overview of MCP-1 roll in Restenosis

It implied that Inflammation has a pathogenic role in the development of restenosis after percutaneous transluminal coronary angioplasty (PTCA). It demonstrated that MCP-1 mRNA was rapidly increased in pig iliac arteries at 2 h up to maximum at 8 h before returning to low levels at 16–24 h after deendothelialization[45]. Hokimoto *et al.* researchs about MCP-1 plasma levels before, 24 and 48 h, and 3 months after elective PTCA for stable angina showed that in the restenosis group Plasma MCP-1 antigen levels were higher at 48 h and 3 months after PTCA in contrast of in the nonrestenosis group[46]. Increasing of MCP-1 level significantly correlate with the monocyte activity increasing, as reflected by enhanced O2– generation. These results showed that in the balloon-injured vessels MCP-1 production and monocyte activation play a basic role in restenosis after PTCA[47].

Cipollone F. and colleagues in 2001 evaluated the role of mcp-1 in restenosis after percutaneous trans-luminal angioplasty (PTCA). They also tested the theory that MCP-1 exerts its effect, at least in part, by inducing O^{2-} generation in circulating monocytes[48]. Mcp-1 plasma levels was measured before PTCA and 1, 5, 15 and 180 days after PTCA in 50 patients (30 male and 20 female with mean age 62 years), those who had ptca surgery and individuals whose angiography was repeated in 6 months follow-up. Restenosis was observed in 12 patients (28%). The change of mcp-1 from baseline level was not observed in patients with or without restenosis. However, during the statistical analysis a significantly increased rate of mcp-1 levels was found after surgery in restenotic patients compared with non-restenotic patients. In contrast, there was no difference in the plasma levels of other chemokine such as RANTES and interleukin-8 between the two groups after ptca. In their study, mcp-1 levels were associated with restenosis. In addition, the increased level of MCP-1 is significantly associated with the increased macrophage activity, which was reflected by O2- generation. Finally, after multivariate regression analysis, the Mcp-1 plasma level was measured 15 days after ptca and showed that Mcp-1 is the only statistically significant independent factor in restenosis. In general, their study shows that probably the mcp-1 production and the accumulation of macrophages in balloon-injured vessel has a central role in restenosis. Mcp-1 may induce the renarrowing of ducts, at least in some cases, with the creation O^{2-} from monocytes[49].

Plasma MCP-1 antigen and restenosis risk after coronary stent implanation

It has been shown that plasma MCP-1 antigen levels were significantly elevated in the patients with stable exertional angina (SEA) compared with the control chest pain syndrome group. It is reported that MCP-1 is upregulated in the primates arteries and in vascular endothelial and smooth muscle cells which exposed to minimally modified lipids[8, 50]; moreover, MCP-1 has a significant role early atherosclerotic lesions development[51, 52]. Recent clinical studies have shown that plasma levels of MCP-1 are increased in patients with unstable angina and acute myocardial infarction[53], so it is logical to conclude that the level of circulating MCP-1 reflects the clinical state of coronary artery disease.

In-stent restenosis continues to be an important clinical issue3,4 and recent studies have indicated that neointimal hyperplasia post stent placement is related to vessel trauma during stent implantation,8–10 although, importantly, angiographic measurements could not predict restenosis post stent implantation.5 In the present study, as well as in the previous report,5 the angiographic measurements did not differ between the patients with restenosis and those without restenosis; however, the current study revealed a difference in the lesion length. Restenosis was present in 17 (41%) of 41 consecutive stent-treated patients, which appears to be a rather high rate for stenting in this size coronary vessels and is thought to be a statistical quirk related to the small number of subjects in the present study.

The researchers showed the serial changes in antigen levels of plasma MCP-1 that had difference between the restenosis group and the non-restenosis group after stent implantation: the MCP-1 level is higher in restenosis group. MCP-1 is expressed by many kinds of cells and modifies the movement of monocytes into an inflammatory lesion in some inflammatory processes.14 researches implied that possible causative factors of restenosis post stent implantation is inflammatory processes. It

demonstrated that MCP-1 promotes neointimal hyperplasia in early neointimal lesion formation and that neutralization of MCP-1 before, and immediately after, arterial injury may be effective in preventing restenosis after angioplasty[41].

Also it shown that heparin infusion therapy can bind with high affinity to a range of cytokines including interferon- gamma and members of the chemokine superfamily, and can block interferon-gamma-dependent upregulation of MCP-1 production by cultured endothelial cells[54, 55].

Antimonocyte chemoattractant protein-1 gene therapy

Recent studies suggested that inflammation is an important determinant of in-stent neointimal hyperplasia. Farb A. described the association of Inflammation with coronary stenting[56, 57]; in early stages neutrophils surround stent struts were observed transiently, whereas chronic inflammatory cells such as monocytes were observed both in early (within 7 days) and late stages (6 months or later) after stenting. Monocyte inflammation increasing is connected with greater neointimal formation after stenting[57,58]. Furthermore, a studies with monoclonal antibody against the adhesion molecule Mac-1 shown a monocyte recruitment and neointimal formation reduction after rabbit iliac artery stenting[59].

As inflammation is inescapable during stent placement, therapies against stent-induced inflammation are a rational approach for stent-associated restenosis reduction. Recent experiments shown that an intense reduction in neointimal formation with rapamycin- eluting stents is mediated by its antiproliferative and anti-inflammatory effects[60].

MCP-1 will rapidly increase in plasma in the first day of coronary intervention[49,55]. Increasing of MCP-1 after angioplasty is a significant and independent predictor of restenosis. transferring of 7ND gene markedly reduced inflammatory and proliferative changes and increased apoptotic cell death, and thus blocked in-stent neointimal hyperplasia.

locally production of MCP-1 induce the recruitment of monocytes and also active lesional monocytes and vascular smooth muscle cells to produce the inflammatory cytokines and growth factors (IL-6, IL-1b, VEGF), which might result in-stent neointimal hyperplasia. 7ND gene transferation suppressed neointimal formation after balloon or cuff injury and in-stent neointimal hyperplasia[61-63].

Horvath et al. exhibited that an antibody injection against murine CCR2 decreased neointimal hyperplasia after iliac arterial stenting in normocholesterolemic cynomolgus monkeys[64].

Although anti-MCP-1 therapy by 7ND gene transfer must be tested for the prevention of restenosis in humans, but its observed effects in rabbit and monkey models imply a consequential potent for this new mode of treatment. MCP-1 protein basal plasma level in patients before PCI are reported to be 500 pg/ml[49]. Other reports show plasma MCP-1 levels in patients to be in the range of 150– 852 pg/ml[55, 65, 66]. Compared to plasma MCP-1 levels in humans, 7ND levels achieved by i.m. transfection of 7ND gene seem to be less.

By attention to Model studies it deduced that MCP-1-mediated inflammation is a basic mediator in the development of experimental restenosis (neointimal formation) after stenting. 7ND gene transfer by next-generation gene therapy may Inhibit stent-associated inflammation and could be a good method to reduce restenosis and to improve clinical outcome after stent placement[67].

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