

Forum Minireview

Stress and Vascular Responses: Anti-inflammatory Therapeutic Strategy Against Atherosclerosis and Restenosis After Coronary Intervention

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Received December 13, 2002; Accepted January 7, 2003

Abstract. Atherosclerosis and restenosis after percutaneous coronary interventions have become major issues in public health in Western countries. Recent studies have revealed that inflammation plays an important role in pathogenesis of cardiovascular diseases. Vascular injury may involve an inflammatory response, which accelerates the recruitment and activation of monocytes through monocyte chemoattractant protein-1 (MCP-1). MCP-1 expression has been shown to be increased in atherosclerotic lesions and balloon injured arteries. Recently, we have devised a new strategy for anti-MCP-1 gene therapy by transfecting mutant MCP-1 gene into skeletal muscle. This mutant MCP-1 has been shown to work as a dominant-negative inhibitor of MCP-1. We here demonstrate that this strategy limited progression of pre-existing atherosclerotic lesions and improved the lesion composition into a more stable phenotype in the hypercholesterolemic mice. This strategy also suppressed monocyte infiltration/activation in the injured site and markedly inhibited restenotic changes (neointimal hyperplasia) in the carotid artery in rabbits, rats, and monkeys after balloon injury or stent implantation. Therefore, MCP-1-mediated monocyte infiltration is essential in the development of restenotic changes as well as atherosclerosis progression. MCP-1 can be a practical therapeutic target for human restenosis and atherosclerosis.

Keywords: monocyte chemoattractant protein-1, monocyte, gene therapy, restenosis, atherosclerosis

Introduction

Percutaneous coronary interventions have now been recognized to be the useful treatment of choice for dilating atherosclerotic stenosis. However, its overall benefits are hampered by restenosis. Although many drugs have been tried, no clinical trials have demonstrated definite benefits to reduce restenosis rate. In addition, atherosclerosis and its complications are the major cause of death in Western countries. Therefore, prevention or treatment of restenosis/atherosclerosis is a major clinical challenge.

It is a well-known fact that atherosclerotic lesions are infiltrated by leukocytes. Recent studies suggest

that the inflammatory response plays an important role not only in atherosclerosis but also in other cardiovascular diseases such as restenosis after balloon angioplasty or stent implantation, arteriopathy after organ transplantations, and vascular remodeling due to hypertension. Chemokines are proinflammatory cytokines, and they regulate migration and infiltration of leukocytes into tissues and subsequently cause their activation. In fact, increased expression of various chemokines in atherosclerotic lesions compared with normal vessels has been reported. About 80% of leukocytes infiltrated in atherosclerotic lesions are monocytes/macrophages, and 10% to 20% of them are memory T lymphocytes. Monocyte chemoattractant protein-1 (MCP-1) is the most important chemokine that regulates migration and infiltration of monocytes/macrophages. MCP-1 belongs to the CC chemokines, a subfamily of

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chemokines. MCP-1 is the specific chemotactic factor for monocytes/macrophages and plays a crucial role in the pathogenesis of chronic inflammation. The effects of MCP-1 are mediated mainly through CC chemokine receptor 2 (CCR2). Recent studies revealed that MCP-1 might play an important role in initiation and pathophysiology of cardiovascular disease. These facts suggest that therapeutic strategies targeting MCP-1 may be effective in treatment of cardiovascular diseases. We have succeeded in developing a new therapeutic strategy that can inhibit MCP-1 effectively *in vivo*. In this review, we describe the role of MCP-1 in atherosclerosis and restenosis after arterial injury and introduce our research to evaluate the efficacy of our anti-MCP-1 gene therapy using mutant MCP-1 gene transfection.

Role of inflammation in atherosclerosis and restenosis

Atherosclerosis is currently recognized to be a chronic inflammatory disease, and migration of monocytes/macrophages is supposed to be an important early event in atherogenesis. MCP-1 is secreted by activated endothelial cells or smooth muscle cells, and it plays an important role in migration and infiltration of monocytes/macrophages into arterial wall (1). Recently, upregulation of MCP-1 in atherosclerotic lesions has been reported (2). Boring et al. crossed CCR2-deficient (CCR2^{-/-}) mice with hypercholesterolemic apolipoprotein E-knockout mice, which develop severe atherosclerosis, and reported that the selective absence of CCR2 markedly decreases atherosclerotic lesion formation in ApoE-knockout (ApoE-KO) mice (3). Namiki et al. transfected the cDNA encoding rat MCP-1 into the vessel wall of the rabbit carotid artery using the hemagglutinating virus of the Japan (HVJ)-liposome method. They reported that all cholesterol fed rabbits displayed neointimal formation with infiltration of RAM-11-positive monocytes, and a part of the lesion also had lipid deposition, although vascular lesion formation was not found in normal chow fed rabbits (4). These data suggest that MCP-1 plays a pivotal role in the development of atherosclerosis.

Inflammation is also implicated in the development of restenosis after angioplasty, and potent role of MCP-1 in the pathogenesis of restenosis has been suggested. Rapid increase of MCP-1 expression after balloon injury has been demonstrated. Cipollone et al. reported that restenotic patients, compared with nonrestenotic patients, had statistically significant elevated levels of MCP-1 after percutaneous transluminal coronary angioplasty (PTCA), and higher MCP-1 throughout the study was correlated with restenosis. Moreover,

increased MCP-1 had significant correlation with increased monocyte activity, and the MCP-1 plasma level measured 15 days after PTCA was a statistically significant independent predictor of restenosis (5). Roque et al. tested the effect of CCR2 deficiency in a murine model of femoral arterial injury. They reported significant reduction in intimal hyperplasia after arterial injury in CCR2^{-/-} mice compared with CCR2^{+/+} mice (6). These data suggest that MCP-1 and their receptor CCR2 may also play a pivotal role in the pathogenesis of restenosis after angioplasty.

Anti-inflammatory therapeutic strategy of mutant MCP-1 gene transfection

We have recently reported that an N-terminal deletion mutant of human MCP-1 (7ND), which lacks the N-terminal amino acids 2 to 8, acts as a dominant negative inhibitor for MCP-1 and blocks the MCP-1/CCR2 signal pathway *in vivo* (7). Rollins et al. reported that this mutant MCP-1 and normal MCP-1 form a heterodimer, which binds to the MCP-1 receptor (CCR2) and completely inhibits MCP-1 mediated monocyte chemotaxis *in vitro* (8) (Fig. 1A). Thus, we employed the new therapeutic strategy targeting MCP-1 using this mutant MCP-1 gene transfection (Fig. 1B). We directly transfected the naked expression plasmid vector encoding the 7ND gene into skeletal muscles, and we demonstrated that 7ND protein was secreted from the transfected skeletal muscle cells into the circulating blood at least for 2 to 4 weeks and subsequently blocked monocyte infiltration into the dermis induced by subcutaneous injection of recombinant MCP-1 (7) (Fig. 1B). On the basis of our these results, we investigated the effect of this strategy on atherosclerosis and restenosis after arterial injury in animal models.

Effects of 7ND transfection on atherosclerosis in ApoE-KO mice

ApoE-KO mice spontaneously develop hypercholesterolemia and atherosclerotic lesions similar to those found in humans (9, 10). We transfected 7ND gene to ApoE-KO mice at 7 to 8 weeks of age, which have not developed apparent atherosclerotic lesions, and evaluate the effect of the transfection on atherogenesis after high cholesterol diet administration. Blockade of the MCP-1 pathway inhibited the formation of atherosclerotic lesions but had no effect on serum lipid concentrations (11). Furthermore, this strategy increased the lesional extracellular matrix content and accordingly, the plaque stability score. These results suggest that MCP-1 is associated with not only atherogenesis but

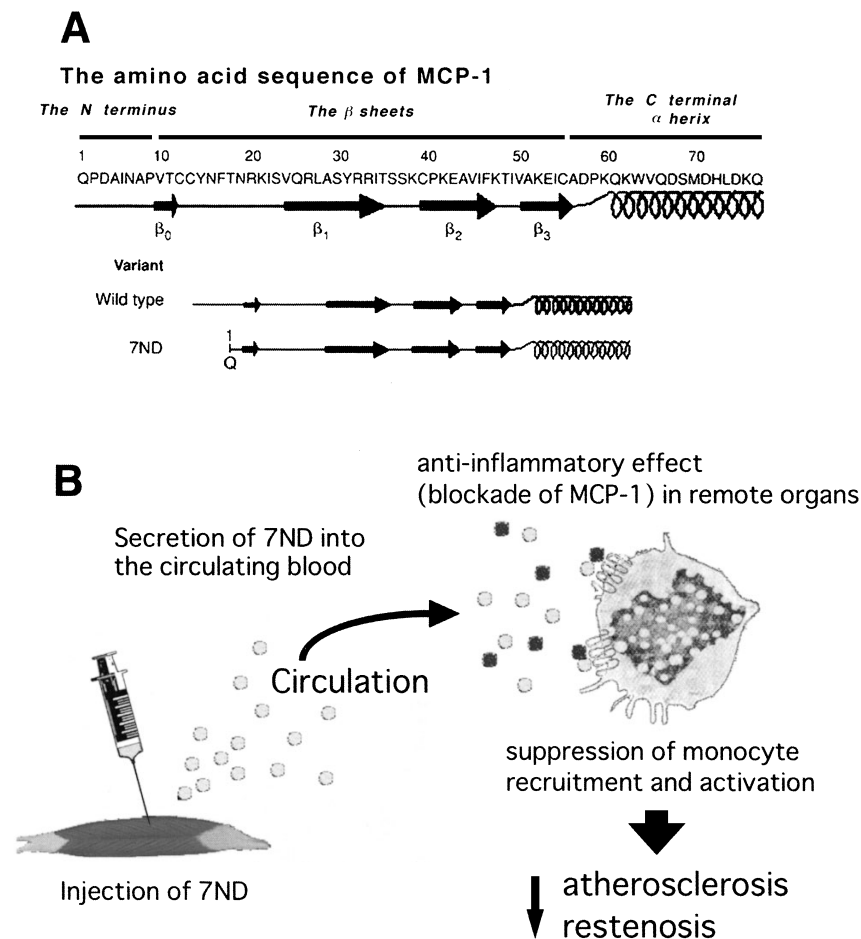


Fig. 1. Structure-functional relationships for MCP-1 (A) and therapeutic strategy of mutant MCP-1 (7ND) gene transfection against atherosclerosis and restenosis after arterial injury (B). A: 7ND is an N-terminal deletion mutant of human MCP-1, which lacks the N-terminal amino acids 2 to 8, and acts as a dominant negative inhibitor for MCP-1. B: We transfected the naked expression plasmid vector encoding 7ND gene into skeletal muscle. 7ND protein is secreted from the transfected skeletal muscle cells into the circulating blood, blocked the MCP-1/CCR2 signal pathway in target vessels, and expected to suppress atherosclerosis or restenosis.

also vulnerable atheromatous plaques stabilization.

We also determined the effect of blockade of MCP-1 on progression of pre-existing atherosclerotic lesions in the aortic root in ApoE KO mice at 20 weeks of age. Blockade of MCP-1 could limit progression of established lesions. In addition, blockade of MCP-1 improved the lesion composition into a more stable phenotype (i.e., containing fewer macrophages and lymphocytes, less lipid, more smooth muscle cells and collagen). This strategy decreased expression of CD40 and the CD40 ligand in the atherosclerotic plaque and normalized the increased chemokine (RANTES and MCP-1) and cytokine (TNF α , IL-6, IL-1 β , and TGF- β 1) gene expression in the aorta (12). Suppression of MCP-1 and the other chemokine and cytokine expression by 7ND gene transfer implies that MCP-1-mediated inflammation creates a positive feedback loop (a vicious cycle)

to enhance vascular inflammation and atherogenesis, possibly through activating lesional monocytes.

These data suggest that MCP-1 is a central mediator in the progression and destabilization of established atheroma, and the inflammatory responses mediated by MCP-1 are important in atherosclerosis and its complications.

Effects of 7ND transfection on restenosis after balloon injury

We also tested the hypothesis that 7ND gene transfection inhibits vascular restenosis after balloon injury or stent implantation. We evaluated the effect of 7ND transfection on the development of restenotic changes after balloon injury in the carotid artery in hypercholesterolemic rabbits. We found that after balloon injury,

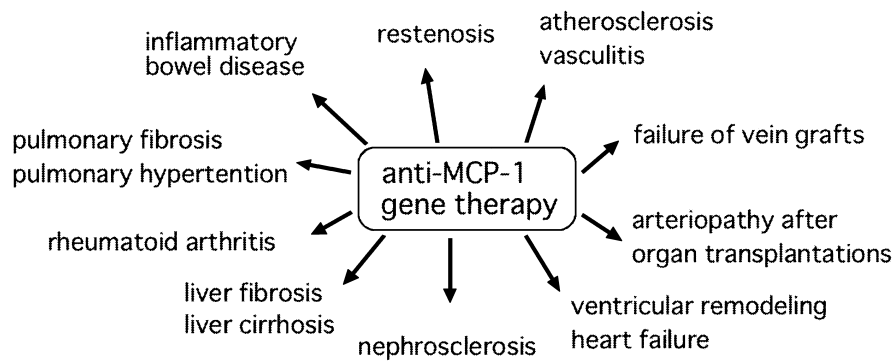


Fig. 2. Clinical application of anti-MCP-1 gene therapy.

MCP-1 mRNA level significantly increased, appearance of RAM11-positive macrophages became evident on days 3 to 7, and neointimal formation and negative remodeling (smaller lumen size, internal elastic lamina, and external elastic lamina) were evident on day 28. Intramuscular transfection of 7ND gene suppressed monocyte infiltration and activation in the injured arterial wall and thus attenuated the development of neointimal hyperplasia as well as negative remodeling (13).

We also assessed the efficacy of this anti-MCP-1 strategy in reducing neointimal hyperplasia after carotid artery balloon injury in other animals including a primate. We observed the increased expression of MCP-1 gene and protein, infiltration of monocytes into the intima, and the appearance of proliferating cells in the early stages (days 3 to 7) of balloon injury in rats. Neointimal hyperplasia was evident in the later stage (day 28). Transfection of mutant MCP-1 gene suppressed such early inflammatory and proliferative changes and inhibited neointimal hyperplasia by 60% (14). Furthermore, this strategy reduced neointimal hyperplasia after balloon injury by 70% (14) and intimal thickening after stent implantation in monkeys (unpublished data).

These data suggest that MCP-1-mediated monocyte infiltration is essential in the development of neointimal hyperplasia after balloon injury and stent implantation in rabbits, rats, and monkeys. This strategy may be a useful form of gene therapy against human restenosis after percutaneous coronary intervention.

Conclusion

Inflammation appears to play an important role in pathogenesis of various diseases. As we described in this review, MCP-1 plays a pivotal role in chronic inflammation of cardiovascular disease, such as athero-

sclerosis and restenosis after vascular injury, by regulating monocyte/macrophage migration and activation. The new anti-MCP-1 therapeutic strategy, which we have developed, may be a useful and feasible gene therapy against arteriosclerosis or restenosis after coronary intervention in humans. Recently, we demonstrated that there was no significant side effect associated with 7ND transfection. We are planning clinical application of this strategy in human restenosis after percutaneous coronary intervention, and this application is under deliberation at the Ministry of Health, Labour and Welfare of the Japanese government. Because our therapeutic strategy does not need local gene transfection into target lesions and can maintain a long efficacy by repeating gene transfection, it is expected that this strategy can be applied to other chronic inflammatory diseases, including pulmonary hypertension, liver fibrosis/cirrhosis, rheumatoid arthritis, etc. (Fig. 2).

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