NLRP3 Inflammasome Inhibition by MCC950 Reduces Atherosclerotic Lesion Development in Apolipoprotein E–Deficient Mice—Brief Report

Thomas van der Heijden, Eva Kritikou, Wouter Venema, Janine van Duijn, Peter J. van Santbrink, Bram Slütter, Amanda C. Foks, Ilze Bot, Johan Kuiper

Objective—Inflammasomes are multiprotein complexes, and their activation has been associated with cardiovascular disease. Inflammasome activation leads to secretion of caspase-1 by innate immune cells, resulting in the activation of interleukin-1β. Recently, a potent and selective inhibitor of the NLRP3 inflammasome, MCC950, was described. In this study, we investigated the effect of MCC950 on atherosclerotic lesion development in apoE$^{-/-}$ mice.

Approach and Results—First, we determined the efficacy of MCC950 in vitro. Bone marrow–derived macrophages and dendritic cells were stimulated with lipopolysaccharide and cholesterol crystals resulting in high levels of interleukin-1β release, which was inhibited by MCC950. In vivo MCC950 treatment reduced lipopolysaccharide–induced interleukin-1β secretion, without affecting the tumor necrosis factor-α response. Subsequently, atherosclerotic plaques were induced in Western-type diet fed apoE$^{-/-}$ mice by semiconstrictive perivascular collar placement at the carotid arteries, after which the mice received MCC950 (10 mg/kg) or vehicle control 3× per week intraperitoneally for 4 weeks. After euthanize, atherosclerotic plaque size and volume were quantified in hematoxylin-eosin–stained 10-μm cryosections throughout the artery. MCC950 treatment significantly reduced the development of atherosclerotic lesions as determined by maximal stenosis, average plaque size, and plaque volume. Although the amount of collagen and the necrotic core size were not affected, the number of macrophages in the plaque was significantly reduced on treatment. In addition, VCAM-1 (vascular cell adhesion molecule 1) and ICAM-1 (intercellular adhesion molecule 1) mRNA expression was significantly reduced in the carotids of MCC950-treated mice.

Conclusions—These findings show that specific inhibition of the NLRP3 inflammasome using MCC950 can be a promising therapeutic approach to inhibit atherosclerotic lesion development.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2017;37: 1457-1461. DOI: 10.1161/ATVBAHA.117.309575.)**

Key Words: atherosclerosis ■ inflammasomes ■ interleukin-1beta ■ macrophages

Inflammasomes are multiprotein complexes that recognize several stimuli, such as damage-associated molecular patterns and pathogen-associated molecular patterns.[1] One of the most extensively described inflammasomes is the NLRP3, which consists of NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain and the cysteine protease caspase-1.[1,2] On activation, the NLRP3 inflammasome can secrete mature forms of caspase-1, which cleave biologically inactive pro-interleukin-1β (IL-1β) into the proinflammatory cytokine IL-1β.[3] Activation of the NLRP3 inflammasome has been associated with cardiovascular disease, as in humans, the severity of coronary artery disease is correlated with NLRP3 expression,[4] and IL-1β levels are increased in atherosclerotic coronaries compared with normal arteries.[5] Furthermore, the expression of NLRP3 inflammasome-related genes is increased significantly in human atherosclerotic lesions compared with nonatherosclerotic vessels.[6]

Also in experimental models, the NLRP3 inflammasome and IL-1β were seen to contribute to atherosclerosis. Cholesterol crystals that accumulate in the plaque are able to activate the NLRP3 inflammasome and induce IL-1β secretion from macrophages after lipopolysaccharide (LPS) priming.[7,8] Reconstitution of LDLr$^{-/-}$ mice with bone marrow lacking either NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain, or IL-1α/IL-1β results in reduced lesion development,[7] and IL-1β deficiency limits atherosclerotic lesion development in apoE$^{-/-}$ mice.[9] Furthermore, it was shown that a monoclonal antibody targeting IL-1β reduced aortic lesion development.[10] Together, these data suggest that NLRP3 inflammasome inhibition may be beneficial in reducing atherosclerotic lesion development.

Recently, a potent and selective NLRP3 inflammasome inhibitor, MCC950, was described.[11] This diarylsulfonylurea-containing compound, of which the chemical structure was described by

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Nonstandard Abbreviations and Acronyms	
ICAM-1	intercellular adhesion molecule 1
IL-1RA	interleukin-1 receptor antagonist
IL-1β	interleukin-1β
VCAM-1	vascular cell adhesion molecule 1

Coll et al,[11] prevents the formation of the NLRP3 inflammasome by inhibiting the NLRP3-induced apoptosis-associated speck-like protein containing a caspase recruitment domain oligomerization. The exact mechanism, however, how inhibition occurs is up to date unknown. MCC950 reduced LPS- and nigericin-induced IL-1β, but not tumor necrosis factor-α release from bone marrow–derived macrophages, thus showing an inflammasome-specific effect.[11] Also in vivo MCC950 blocks NLRP3 activation[11] and was recently shown to reduce myocardial infarct size.[12] Therefore, in this study, we treated apoE$^{-/-}$ mice with MCC950 for 4 weeks to establish its effects on atherosclerosis.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Bone marrow–derived macrophages and bone marrow–derived dendritic cells were stimulated in vitro with LPS and cholesterol crystals in the presence of 1 μmol/L MCC950 to determine its efficacy. Combined LPS and cholesterol crystals stimulation resulted in a robust release of IL-1β by bone marrow–derived macrophages (Figure 1A; $P<0.001$) and bone marrow–derived dendritic cells (Figure 1B; $P<0.001$), which was completely inhibited by MCC950 treatment (Figure 1A and 1B; $P<0.001$). Tumor necrosis factor-α secretion was not affected (Figure IA and IB in the online-only Data Supplement), illustrating an NLRP3 inflammasome–specific effect. To determine the efficacy of MCC950 in vivo in a hyperlipidemic environment, apoE$^{-/-}$ mice on a Western-type diet were challenged with LPS after 1 week of MCC950 treatment. MCC950 treatment resulted in a strong reduction in the IL-1β secretion (Figure 1C; $P<0.001$) without affecting serum tumor necrosis factor-α levels (Figure IC in the online-only Data Supplement), indicating that MCC950 treatment is also effective under hyperlipidemic conditions.

Next, we investigated the effects of NLRP3 inhibition on atherosclerotic lesion development by treating apoE$^{-/-}$ mice with MCC950 for 4 weeks. During the study, MCC950 treatment did not affect body weight, serum triglyceride, or glucose levels (Figure IIA through IIC in the online-only Data Supplement). There was also no difference in serum total cholesterol during the study, and, although there was a small but not significant reduction in the very-low-density lipoprotein peak, there were no differences in total very-low-density lipoprotein, low-density lipoprotein, or high-density lipoprotein levels as determined by lipoprotein analysis (Figure IID through IIF in the online-only Data Supplement). MCC950 treatment reduced plaque size at the site of maximal stenosis from $1.16\pm0.09\times10^5$ μm^2 in control mice to $0.88\pm0.06\times10^5$ μm^2 in MCC950-treated mice (Figure 1D; $P<0.05$). The average plaque size was reduced by 26% from $0.71\pm0.07\times10^5$ μm^2 in control mice to $0.52\pm0.05\times10^5$ μm^2 in MCC950-treated mice (Figure 1D; $P<0.05$). Similarly, plaque volume was reduced ($0.84\pm0.18\times10^8$ versus $0.37\pm0.05\times10^8$ μm^3; Figure 1D; $P<0.05$). Lipid content, as measured by an Oil-Red-O staining, was also reduced in MCC950-treated mice (Figure III in the online-only Data Supplement).

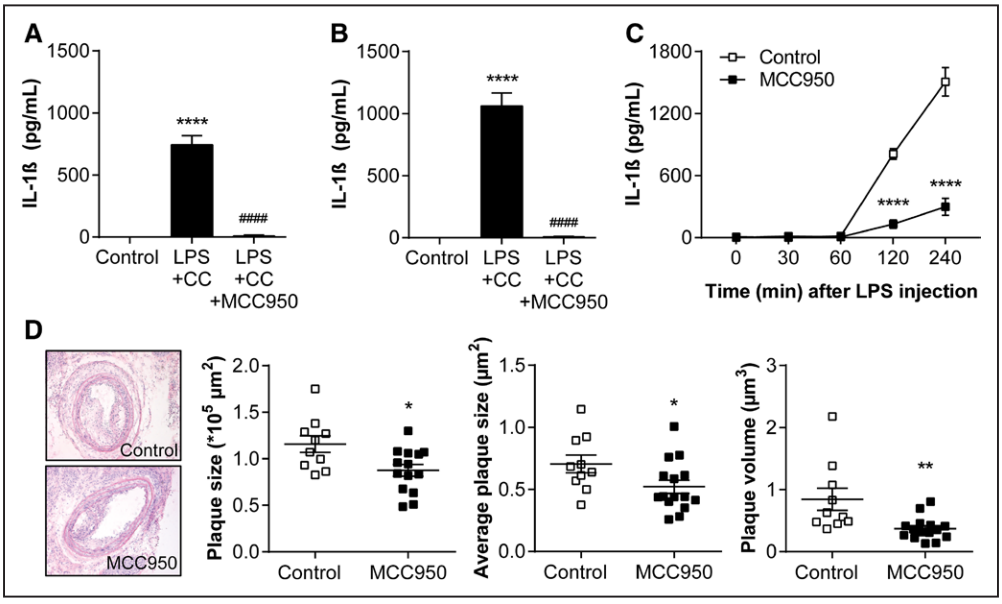

Figure 1. Analysis of NLRP3 inflammasome inhibition by MCC950 in vitro showed that interleukin-1β (IL-1β) secretion on stimulation with lipopolysaccharide (LPS) and cholesterol crystals (CC) was inhibited by MCC950 in both (**A**) bone marrow–derived macrophages (BMDMs) and (**B**) bone marrow–derived dendritic cells (BMDCs). **C**, Also in vivo, IL-1β secretion in plasma was reduced by MCC950 on LPS challenge of Western-type diet fed apoE$^{-/-}$ mice. **D**, MCC950 treatment significantly reduced carotid artery plaque size (magnification ×100, left graph: plaque size at the site of maximal stenosis, middle graph: average plaque size, right graph: plaque volume). (*$P<0.05$, **$P<0.01$, ****$P<0.0001$ compared with control, ####$P<0.0001$ compared with LPS+CC).

Macrophage content as measured by a MOMA-2 staining was reduced, both in absolute values (−42%, controls: 46.38±5.07×10^3 μm^2; MCC950: 26.79±2.12×10^3 μm^2; Figure 2A; P<0.05) and relative to plaque area (−24%, controls: 39.0±1.7%; MCC950: 29.8±2.7%; Figure 2A; P<0.05). The relative necrotic core area and collagen content did not differ between the groups (Figure 2B and 2C). In addition, we did not observe differences between groups in the number of perivascular mast cells (and its activation status) and perivascular neutrophils in the carotid arteries (data not shown).

NLRP3 inflammasome inhibition by MCC950 reduced the amount of circulating white blood cells with 29% (Figure IVA in the online-only Data Supplement; P=0.08), which was primarily because of a reduction of 43% in circulating neutrophil numbers (Figure IIIB in the online-only Data Supplement; P<0.05). Circulating monocyte or CD4/8 T-cell levels were not affected (Figure IVC through IVE in the online-only Data Supplement). Also, IL-18 and serum amyloid A levels did not differ between the groups (Figure IVF and IVG in the online-only Data Supplement), whereas IL-1β and IL-6 levels were not detectable.

Treatment with MCC950 did not affect mRNA expression of NLRP3 or that of NLRP3-related genes in the carotid arteries (Figure 2D). In line with previous studies, in which the lack of IL-1β reduced aortic mRNA expression levels of the adhesion molecules VCAM-1 (vascular cell adhesion molecule 1) and ICAM-1 (intercellular adhesion molecule 1),[9] we did observe a significant reduction in the expression of both VCAM-1 and ICAM-1 in the carotids on MCC950 treatment (Figure 2D; P<0.05), whereas local MCP-1 (monocyte chemoattractant protein-1) expression levels were not affected. These observations suggest that MCC950, by inhibiting the NLRP3 inflammasome, can reduce the influx of macrophages and thereby atherosclerotic lesion development.

Discussion

Activation of the NLRP3 inflammasome leads to increased levels of active IL-1β, which can promote the development of atherosclerosis.[8–10,13] Mice lacking either NLRP3 or IL-1β display reduced atherosclerosis, and blockade of the IL-1 receptor results in decreased lesion size.[7,9,14] Therefore, inhibition of the NLRP3 inflammasome could be a potential therapeutic strategy to treat atherosclerosis. Here, we show that a small

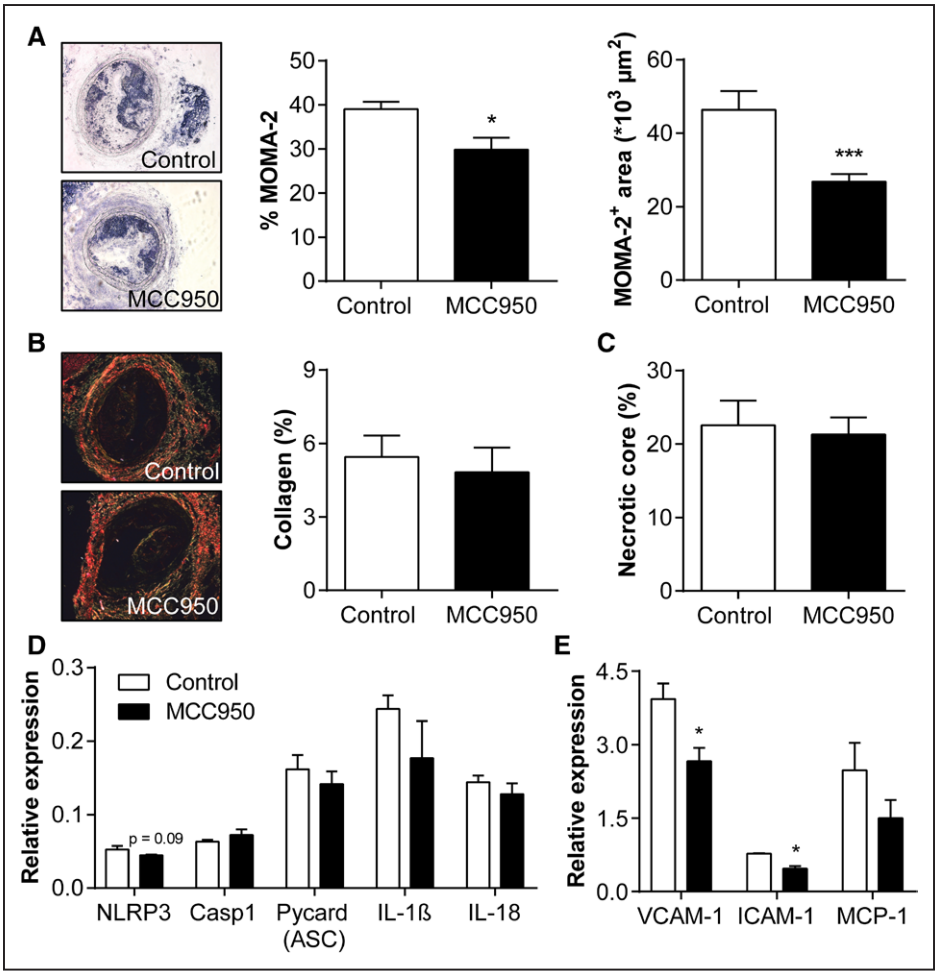

Figure 2. MCC950 treatment of apoE$^{-/-}$ mice resulted in (**A**) reduced plaque macrophage content, both relative to plaque size and in absolute area, but did not affect collagen content (**B**) or necrotic area (**C**) of the carotid artery plaques (magnification ×100). Gene expression analysis of these carotid artery plaques established that MCC950 treatment did not affect NLRP3 inflammasome–related genes (**D**), but reduced the expression of the adhesion molecules VCAM-1 (vascular cell adhesion molecule 1) and ICAM-1 (intercellular adhesion molecule 1; **E**; *P<0.05, ***P<0.001 compared with control). ASC indicates apoptosis-associated speck-like protein containing a caspase recruitment domain.

molecule inhibitor of the NLRP3 inflammasome, MCC950, reduces atherosclerotic lesion development and the influx of macrophages in the carotid artery. Recently, MCC950 was shown to prevent IL-1β secretion on NLRP3 stimulation both in vitro and in vivo,[11] and we have now established the efficacy of this compound under hyperlipidemic conditions.

In this study, we show that MCC950 reduced atherosclerotic lesion development, which mainly resulted from a reduction in the number of macrophages inside the lesion. Previously, it was established that lack of IL-1β reduced the expression of adhesion molecules VCAM-1 and ICAM-1 in the aorta of apoE$^{-/-}$ mice,[9] whereas IL-1β exposure induced the expression of these molecules in human vascular smooth muscle cells in vitro.[15] Also, deficiency of IL-1RA (interleukin-1 receptor antagonist) in apoE$^{-/-}$ mice resulted in highly increased expression of ICAM-1, VCAM-1, and MCP-1 in the aorta.[16] In line with these data, we observe that inhibition of the NLRP3 inflammasome by MCC950, and thereby IL-1β secretion, reduces both VCAM-1 and ICAM-1 mRNA expression in the carotid artery. This may have resulted in reduced monocyte adhesion to the vessel wall and to a subsequent decrease in intraplaque macrophages. However, MCC950 may have affected intraplaque macrophage content indirectly, or via other mechanisms involved such as by affecting macrophage proliferation or retention in the lesion. Future research may shed more light on the exact mechanisms via which MCC950 reduces macrophage numbers in the plaque. Systemically, MCC950 reduces circulating neutrophils, but we do not observe differences in the number of neutrophils in the perivascular tissue. Although IL-1 signaling has shown to be involved in neutrophil recruitment, this was mostly observed in acute inflammation models by injecting (cholesterol) crystals intraperitoneally.[7,17] Furthermore, a previous study showed that IL-1α induces the recruitment of neutrophils, whereas IL-1β primarily promotes the recruitment of macrophages.[18]

Interestingly, MCC950 can also inhibit human NLRP3. Processing of both IL-1β and caspase-1 in isolated peripheral blood mononuclear cells from human subjects was dose dependently inhibited by MCC950.[11] In human atherosclerotic lesions, the expression of NLRP3 and related genes was shown to be upregulated compared with nonatherosclerotic vessels,[6] and also, IL-1β levels are increased in human atherosclerotic coronary arteries.[5] By inhibiting the NLRP3 inflammasome, MCC950 reduces IL-1β secretion and could be of therapeutic use to cardiovascular patients. IL-1β inhibition by canakinumab, for example, has shown to reduce IL-6 and hs-CRP (high-sensitivity C-reactive protein) levels in both men and women with well-controlled diabetes mellitus and high cardiovascular risk.[19] Canakinumab also reduced IL-6 and hs-CRP levels in patients with atherosclerotic disease, but no improvements of the vascular structure or function were found thus far.[20] Of note, patients treated with canakinumab had increased levels of total cholesterol, and this study did not investigate the effects on plaque inflammation or leukocyte function.[20] In contrast to this study, we do not observe an increase in serum total cholesterol levels using MCC950. Currently, the effect of IL-1β inhibition by canakinumab on reducing recurrent myocardial infarction, stroke, and cardiovascular death is investigated in stable patients with coronary artery disease.[21] Further studies analyzing the effect of MCC950 in other experimental models of atherosclerosis to confirm our findings, and specifically on advanced atherosclerosis for human relevance, would thus be highly recommended.

In conclusion, we show that MCC950 inhibits atherosclerotic lesion development, presumably via a reduction in adhesion of monocytes leading to a reduced intraplaque macrophage content. On the basis of our findings, blocking the NLRP3 inflammasome pathway by MCC950 could be a novel strategy to inhibit atherosclerotic lesion development.

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Disclosures

None.

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Highlights

- Interleukin-1β secretion on stimulation with lipopolysaccharide and cholesterol crystals was inhibited by MCC950 in both bone marrow–derived macrophages and bone marrow–derived dendritic cells.
- MCC950 treatment inhibits atherosclerotic lesion development and reduced intraplaque macrophage content in apoE$^{-/-}$ mice.
- MCC950 reduced VCAM-1 (vascular cell adhesion molecule 1) and ICAM-1 (intercellular adhesion molecule 1) mRNA expression in the atherosclerotic plaque.