Micelles selectively target atherosclerosis plaques through peptide-based targeting

Review Article

Atherosclerotic cardiovascular disease leads to myocardial infarction and stroke, making it one of the major causes of premature death worldwide. The root cause of atherosclerosis, which is an inflammatory disease, is the build up of lipid-rich plaque on artery walls. This is the result of a cascade of events triggered by the accumulation of cholesterol particles in the arterial wall. This stimulates the activation of endothelial cells, leading to massive recruitment of monocytes that differentiate into macrophages, escalating the local inflammatory response and finally resulting in plaque formation. To enable early detection and analysis of plaque formation, researchers at the Institute for Molecular Engineering, University of Chicago, USA, have constructed monocyte-targeting peptide amphiphile micelles (PAMs). Adding a chemokine receptor-binding motif enables the micelles to selectively target monocytes, making them promising tools in both diagnostic and preventive medicine.

Monocytes for the early detection of plaque formation

Imaging technologies for atherosclerosis, including intravascular ultrasound, computer tomography and magnetic resonance imaging have a number of disadvantages, such as low sensitivity, limited accuracy and reproducibility, and poor cost-efficiency. Most importantly, these technologies do not enable correlation of physical traits such as the thickness of the plaque's fibrous cap, or relative area of the necrotic core to the molecular state of the plaque. Targeting monocytes could provide a molecular imaging tool that could enable early detection of plaque formation, quantify its extent, and identify the plaques most likely to lead to myocardial infarction or stroke.

Targeting monocytes through MCP-1

The activation of endothelial cells early in plaque formation includes secretion of monocyte chemoattractant protein-1 (MCP-1, also known as C–C chemokine ligand 2). This is a chemokine that binds to the C–C chemokine receptor, CCR2, on the monocyte surface, resulting in massive recruitment of monocytes.

The researchers at the Institute for Molecular Engineering, University of Chicago, therefore decided to construct monocyte-targeting PAMs based on the CCR2-binding motif (residues 13–35) of MCP-1, YNFTNRKISVQRLASYRRITSSK. They modified this peptide by adding a cysteine residue to the N-terminus to enable conjugation via a thioether linkage to a hydrophobic distearoyl lipid tail (1,2-distearoyl-snglycero-3-phosphoethanolamine, or DSPE) with a PEG linker. The construct 'MCP-1 peptide – PEG – lipid tail' readily forms uniform small spherical micelles that are optimal for intravascular injection (Figure 1).

Control, non-targeting PAMs were based on a scrambled version of the MCP-1 sequence. The researchers also constructed Cy7-labeled fluorescent micelles for deep tissue imaging studies with low auto-fluorescence. These micelles were prepared by mixing lipid tail–PEG–Cy7 with either lipid tail–PEG–MCP-1 or lipid tail–PEG–scrambled.

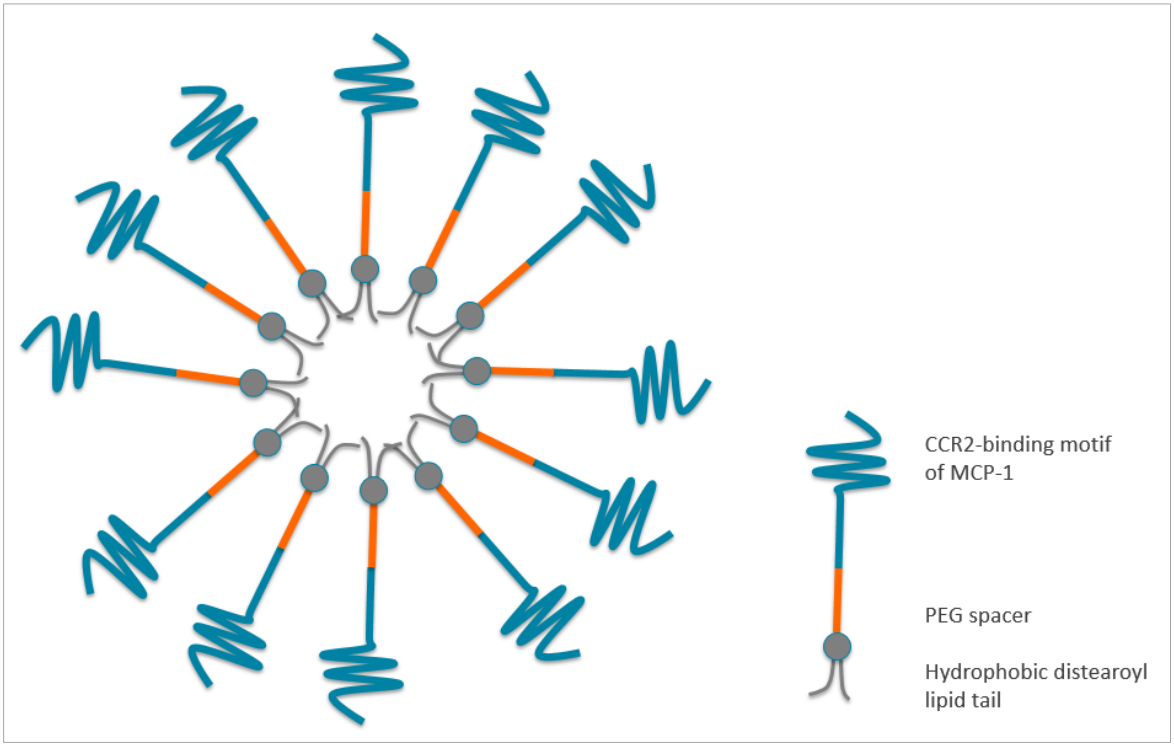

Figure 1. *The construct 'MCP-1 peptide – PEG – lipid tail' readily forms uniform small spherical micelles. Based on Figure 1A, Chung et al 2015.*

Synthesis of the CCR2-binding motif of MCP-1 and micelle formation

Peptides were synthesized with standard Fmoc-mediated solid-phase peptide synthesis methods on a Wang resin using a PS3 Automated Solid Phase Peptide Synthesizer. A cysteine residue was added to the N-terminus of the CCR2-binding motif (residues 13–35) of the MCP-1 protein [YNFTNRKISVQRLASYRRITSSK] and scrambled peptide sequence [YNSLVFRIRNSTQRKYRASIST] to enable covalent conjugation to the micelle lipid tail. Peptides were deprotected, cleaved, precipitated and washed several times before lyophilization. Crude peptide mixtures were purified by reverse-phase HPLC and characterized by MALDI-TOF/TOF. The cysteine-containing peptides were conjugated via a thioether linkage to DSPE-PEG2000-maleimide and then purified and characterized. Cy7-labeled molecules were prepared by conjugation via an amide bond by adding Cy7 mono-N-hydroxysuccinimide ester to the lipid DSPE-PEG2000-amine.

The researchers assembled the fluorescently-labeled MCP-1 micelles by dissolving the Cy7- and peptide-containing DSPE-PEG(2000) amphiphiles in methanol, mixing the components, and evaporating under nitrogen. They dried the film under vacuum, hydrated it at 80 °C and cooled to room temperature.

Shifts in secondary structure after micelle formation

The secondary structure of the peptides shifted after micelle formation, with increased levels of β-sheet and decreased levels of random coil structures. The recognition of MCP-1 PAMs by the CCR2 receptor and cell membrane interactions depend on both peptide sequence and secondary structure. The PEG spacer drives spherical micelle formation by swelling the corona, but does not dictate peptide secondary structure. The overall micelle interactions might be enhanced by further stabilizing specific secondary structures.

PAMs can detect and differentiate stages of atherosclerosis

Eun Chung and colleagues used a murine ApoE knock-out model to investigate the targeting of Cy7-MCP-1 PAMs to monocytes and showed that the level of PAM binding increased as disease progressed (Figure 2). They could confirm the specificity of the binding by *ex vivo* imaging. Histological studies indicated that the PAMs accumulated mostly on the luminal surface of the vessel wall in early stage atherosclerosis and on plaques in later stages, which corresponds with increased expression levels of CCR2.

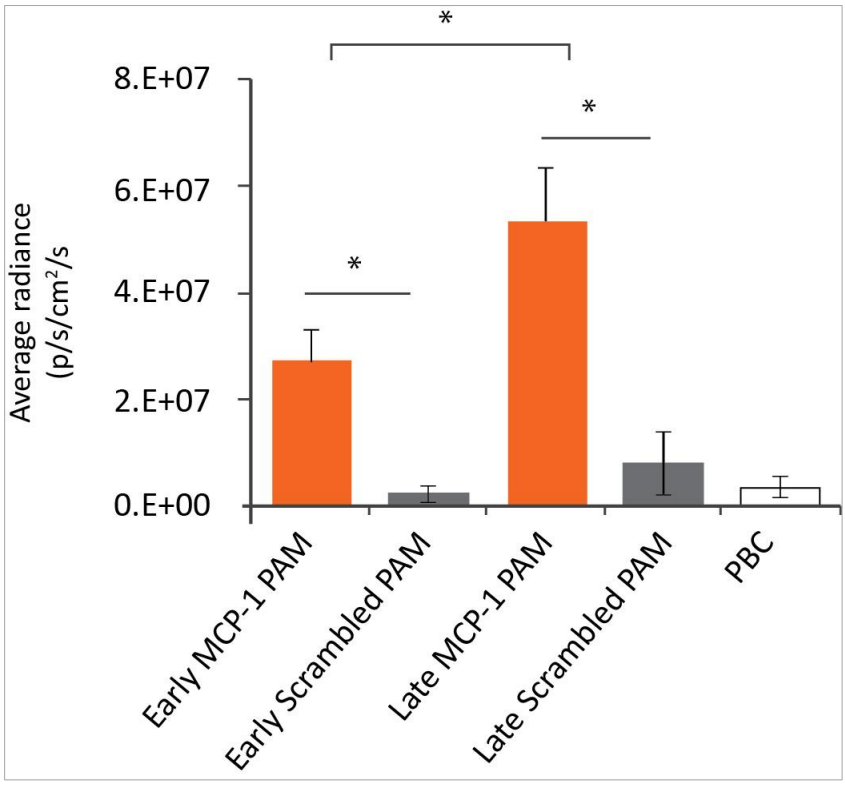

Figure 2. *The specificity of the Cy7 MCP-1 PAMs for atherosclerotic plaques was clear, with significantly higher levels binding to aortas at an advanced stage of atherosclerosis compared to those at an early stage. Data points are mean ±SD, * P < 0.05, N ≥ 3 mice. Redrawn from Figure 3B, Chung et al, 2015.*

MCP-1 PAMs confirmed as safe

After accumulation in the bladder, liver and kidney, micelles were cleared in seven days, and there were no signs of tissue or cellular damage. The MCP-1 PAMs did not appear to be cytotoxic since levels of the apoptosis marker, caspase-3, were comparable to controls.

A promising tool for both diagnostic and preventive medicine

This proof-of-concept clearly demonstrates that intravenously administered MCP-1 PAMs can target plaques in a mouse model and can be used to quantitate the progression of atherosclerosis without damaging vital organs. The small size of the micelles enables them to interact with receptor targets with high specificity and avidity and avidity and they provide a high surface area for targeting ligands together with imaging agents for tracking. The authors concluded, *"As the accumulation of monocytes is linked to atherosclerosis progression, MCP-1 PAMs are a promising tool for both diagnostic and preventive medicine, and have the potential to be developed as theranostic systems for a variety of diseases where inflammation is the underlying cause."*

Reference

Monocyte-targeting supramolecular micellar assemblies: a molecular diagnostic tool for atherosclerosis. Chung EJ, et al. Adv Healthc Mater. 2015 Feb 18;4(3):367-76. doi: 10.1002/adhm.201400336. Epub 2014 Aug 22.