Medical Hypotheses 102 (2017) 19–22

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Nanoparticles as agents targeting cholesterol crystallization in atherosclerosis

Yana Anfinogenova [a,b,c,*], Maria Shvedova [b,c], Sergey V. Popov [a]

[a] Cardiology Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, Tomsk, Russia
[b] National Research Tomsk Polytechnic University, Tomsk, Russia
[c] Siberian State Medical University, Tomsk, Russia

ARTICLE INFO

Article history:
Received 21 September 2016
Accepted 5 March 2017

Keywords:
Nanoparticles
Atherosclerosis
Cholesterol crystals

ABSTRACT

This article introduces a hypothesis on nanoparticle-mediated modulation of cholesterol crystal behaviour in the atherosclerotic plaques. The role of cholesterol crystals in progression of atherosclerosis is emphasized. Proposed mechanism of spontaneous cholesterol crystal formation in the organism is discussed. Mechanisms and factors associated with the nanoparticle-mediated modulation of cholesterol crystal behaviour are proposed. Authors hypothesize that specially designed nanoparticles may therapeutically modulate cholesterol crystal behaviour in atherosclerosis. Nano-sized agents used in stent coatings and imaging techniques can possibly prevent cholesterol crystallization in the diseased vessels. On the other hand, new nanotechnologies should be implemented with caution as certain types of nanoparticles could become crystal seeds for cholesterol deposited in the atherosclerotically damaged vascular walls causing destabilization of the plaques. Studying nanoparticle-induced alterations of cholesterol crystal formation requires multidisciplinary approach involving biomedical researchers, computer scientists, and physical chemists specializing in crystal growth. The proposed hypothesis on nanoparticle-mediated modulation of cholesterol crystal behaviour may be relevant to other medical conditions including gallbladder stones, arthritis, and ophthalmological diseases such as synchysis scintillans.

Endogenous macromolecules represent naturally occurring nano-sized systems with complex functions fully integrated in sophisticated ensembles of the living cells and beyond. For scientists, it is hard to compete with nature in creating artificial nano-sized bioactive agents of comparable functionality, sophistication, and harmony. Nevertheless, chemically and physically diverse nanoparticles have been created to improve tissue functioning. Recent advances have illustrated a potential of nanoparticle-based agents for the diagnosis and treatment of atherosclerosis, one of the primary causes of death and disability throughout the world. Extensive research has been done in this regard in cardiology. However, there is an understudied area of research where nanoparticles can be especially valuable and where nanoparticle research promises a lot of novelty. We hypothesize here for the first time that nanoparticles can modulate the behaviour of cholesterol crystals playing a crucial role in the pathogenesis of atherosclerosis, cholesterol crystal embolism, and some other diseases. Nanotechnology targeting cholesterol crystallization *in vivo* may be especially helpful in the field of interventional cardiology where cholesterol crystal embolism and cholesterol crystal formation within the atherosclerotic plaques sometimes complicate catheter-based interventions in adults. Solving this challenge may contribute to a significant breakthrough considering growing number of catheter-based cardiovascular interventions worldwide. On the other hand, some nanoparticle-based formulations could act as crystal nucleation agents accelerating cholesterol crystal formation within the atherosclerotic plaque and causing its destabilization.

Cholesterol crystal formation in atherosclerosis

Cholesterol crystal formation is involved in the pathogenesis of atherosclerosis [1–8] and a number of hepatic, rheumatologic, and ophthalmologic disorders [9–11]. In atherosclerosis, onset of acicular cholesterol crystal growth causes cell damage, rupture of atherosclerotic plaque fibrous cap, arterial thrombosis, myocardial infarction, or stroke. A crystalline form of cholesterol is a trigger for production of the proinflammatory cytokine IL-1β and it is the link between inflammation and atherosclerosis [9,12]. Sharp-tipped

* Corresponding author at: 111a Kievskaya Street, Tomsk 634012, Russia.
E-mail address: anfiyj@gmail.com (Y. Anfinogenova).

http://dx.doi.org/10.1016/j.mehy.2017.03.002
0306-9877/

edges of cholesterol crystals can cut through fibrous tissues [4,5]. Quickly growing acicular cholesterol crystals penetrate membranes of the surrounding cells triggering the release of biologically active substances and proteolytic enzymes further damaging surrounding tissues. Once a saturated solution of cholesterol develops in the core, crystallization is initiated and occurs suddenly and irreversibly [7,8] in a phase transition-like manner. Quick formation and release of cholesterol crystals from the atherosclerotic plaques can cause life-threatening embolism and delayed-onset paraplegia after cardiovascular surgery [13].

Little is known about the factors triggering nucleation and growth of cholesterol crystals in an atherosclerotic plaque and it is typically difficult to experimentally study nucleation and crystal formation, especially *in vivo*. Considering that cholesterol crystal formation may be an irreversible process *in vivo*, it is essential to understand how it occurs and how to attenuate or completely prevent it. Nucleation is the first step in the formation of crystal via self-assembly or self-organization. Heterogeneous nucleation occurs on phase interfaces whereas homogeneous nucleation occurs away from a surface of a phase interface. Findings, obtained by using exogenous cholesterol and a monoclonal antibody that specifically recognizes ordered cholesterol arrays, demonstrate that three-dimensional (3D) cholesterol crystals can undergo directed nucleation from bilayer membranes containing two-dimensional (2D) cholesterol crystalline domains *in vitro*. 3D crystals are formed exclusively on the bilayer regions where there are segregated 2D cholesterol crystalline domains and they form on the domains [14].

The key factors affecting the interfacial free energy for nucleation of any crystal in liquids include shear flow [15] and trace additives [16]. Shear flow is present in the atherosclerotic plaque domains exposed to intravascular space. In case of cholesterol crystallization in atherosclerosis, trace additives could play an important role when nanomedicines such as imaging agents are used or nanoparticle-eluting vascular stents are placed to restore arterial patency.

Hypothesis: Nanoparticles affect cholesterol crystal behaviour

Nanoparticles have been shown to selectively target cholesterol crystals in cardiovascular imaging research [17]. However, the idea that nanoparticles can therapeutically modulate cholesterol crystal behaviour in atherosclerotic plaques has been underinvestigated perhaps because methodology of studying cholesterol crystals is still poorly developed. Indeed, cholesterol crystals are dissolved by many fixatives in the process of histology slide preparation and only clefts are left in the affected tissues on the slides. It is even more challenging to study cholesterol crystals *in vivo*.

We hypothesize that nanoparticles functionalized by molecules with high affinity to cholesterol can prevent crystallization of amorphous cholesterol in the atherosclerotic plaques without disrupting surrounding cells. Preliminary studies demonstrate that hybrid organic-inorganic nanoparticles accumulate in cholesterol deposited in atherosclerotic plaques [18]. Several formulations obtained through covalent modification of carbon-coated Fe-core nanoparticles by arenediazonium tosylates have been used [19] to characterize patterns of nanoparticle interactions with human atherosclerotic plaque material isolated from cardiovascular patients during surgery. Exposure of isolated human atherosclerotic plaque to surface-modified hybrid nanoparticles results in nanoparticle accumulation within plaque material where it reaches cholesterol deposits and changes physical-chemical properties of free cholesterol [18].

The experimental verification and the models for nanoparticle-mediated modulation of cholesterol crystal formation in native tissue environment are currently unavailable. Interactions of nanoparticles with the lipid rafts of the cells can significantly affect implications of the hypothesis for nanoparticle-induced modulation of cholesterol crystals, but these interactions are also still poorly understood especially in the cells of cardiovascular system. Experimental studies of the cells from other tissues suggest that lipid rafts interact with nanoparticles and are involved in the mechanisms of nanoparticle internalization and transport across the cells depending on particle size and surface charge [20,21]. Lipid rafts rich in cholesterol can be a target of nanoparticles designed to prevent crystallization of free cholesterol. Dimensional characteristics, dynamics, regulation, and functions of lipid rafts in various cell types in cardiovascular system require further studies.

Supramolecular properties of nanoparticles can differentiate their effects on cholesterol-rich structures

Cholesterol-rich formations comprise physiological and pathological structures. Pathological structures include free cholesterol deposited in the atherosclerotic plaques and cholesterol crystals in the various organs and tissues. Lipid rafts represent physiological cholesterol-rich formations. The size of nanoparticles with high affinity to cholesterol can differentiate their effects on these targets.

The smaller the diameter of hydrophobic nanoparticles with high affinity to cholesterol is, the more likely these nanoparticles directly interact with lipid bilayer of the cell membrane especially within the lipid rafts rich in cholesterol. Cell membrane contains integral and semi-integral proteins exposing their hydrophilic domains to the extracellular space. Together with glycocalyx, these hydrophilic domains protect hydrophobic components of the cell membrane from direct contact with hydrophobic particles whose dimensions are large enough. There may be a threshold size of hydrophobic nanoparticles preventing their interaction with the lipid rafts, but still permitting to interact with free cholesterol deposits in atherosclerotic plaques.

Interactions of lipid rafts with nanoparticles designed to target free cholesterol can control both clearance of these nano-agents and the side effects. Short ligand surface functionalization strongly affects modes of lipid raft-dependent uptake/elimination of nanoparticles. Clearance of nanoparticles that do not interact with lipid rafts is significantly slowed down or abrogated [22]. Further modelling and experimental studies of the interactions between various types of nanoparticles, cholesterol deposits, and lipid rafts of the cells are of high demand.

Supramolecular properties [23] will enable nanoparticles to prevent cholesterol crystallization if (i) cholesterol molecules will have higher affinity to molecules covering nanoparticles than to each other; (ii) surface modifying molecules will form covalent bonds with the rigid-core nanoparticles. Such mesostructured assemblies can display useful functional behaviour by disrupting short- and long-range order in liquid and solid cholesterol crystals through affecting crystal bond lengths, coordination numbers, and bond angle ranges. It is theoretically possible because attractive forces of covalent bonds are stronger than weak intermolecular forces building short- and long-range order in cholesterol crystals. Such nanoparticles will create new order of molecules in free cholesterol deposited in atherosclerotic plaques.

Developing such nanoparticles may lead to breakthrough in the management of atherosclerosis and other diseases where acicular cholesterol crystal growth plays a significant role in pathogenesis. It requires interdisciplinary collaboration of research teams. Considering that super-computers become increasingly available for diverse scientific community, developing of the models elucidating