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REVIEW ARTICLE

Nanotechnological Strategies as Smart ways for Diagnosis and Treatment of the Atherosclerosis

Virna M. Martín Giménez[1], Alejandra Beatriz Camargo[2,3], Diego Kassuha[1] and Walter Manucha[4,5,*]

[1]*Instituto de Investigación en Ciencias Químicas, Facultad de Ciencias de la Alimentación, Bioquímicas y Farmacéuticas, Universidad Católica de Cuyo, San Juan, San Juan, Argentina;* [2]*IBAM, UNCuyo, Conicet, Facultad de Ciencias Agrarias, Chacras de Coria, Luján, Mendoza, Argentina;* [3]*Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Mendoza, Argentina;* [4]*Instituto de Medicina y Biología Experimental de Cuyo, Consejo Nacional de Investigación Científica y Tecnológica (Imbecu-Conicet), Argentina;* [5]*Laboratorio de Farmacología Experimental Básica y Traslacional, Área de Farmacología, Departamento de Patología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo, Mendoza, Argentina.*

Abstract: Atherosclerosis provokes a continuous worsening of affected vessels causing a blood flow diminution with several complications and with clinical manifestations that generally appear in advanced phases of the illness. Hence, the conventional therapies are not enough because the atherosclerotic injuries are often irrevocable. For this reason, emerges the necessity to implement smart ways of drug supply and develop new therapeutic targets that decrease the advance atherosclerotic lesion. It results due to particular interest to use new tools for prevention, diagnosis, and treatment of this cardiovascular disease, thus concentrating our attention to accomplish better management on the immune system. Finally, this mini-review highlights the most recent knowledge about nanotechnology as a robust, novel and promissory therapeutic option applied to atherosclerotic pathology, nevertheless, we also alert for possible issues associated with their use.

1. INTRODUCTION

The prevalence of atherosclerosis has increased steadily worldwide due to the increasing elderly population and diet habits rich in saturated fats and decreased physical activity [1]. Additionally, new non-traditional risk factors for atherosclerosis have been identified that share as a common denominator for the activation of the immune system. These findings establish inflammation as an essential element in the initiation, progression, and destabilization of atherosclerotic plaques [2-7].

Atherosclerotic disease is characterized by arteries with thickened and rigid walls, deregulation of lipid metabolism and natural plaque formation. Here, the restriction of blood flow and the eventual rupture of the plaque itself can lead to lethal events [8]. Plaque formation is a complicated biological process that includes endothelial dysfunction, infiltration of macrophages, expression of inflammatory factors, neovascularization, remodeling of the intima and media, among the most prominent phenomena [9].

Macrophages abound within the atherosclerotic plaque, which, together with monocytes, participate in the initiation, progression, and destabilization of the atherosclerotic plaque by various mechanisms. In the early stages, macrophages contribute to the elimination of reactive particles of oxidized low-density lipoprotein (LDLox) through a scavenger receptor (SR-A) and uptake mediated by CD36 cells. In later stages, macrophages are affected in their phagocytic functionality due to the intracellular accumulation of LDLox. Apoptosis eliminates the macrophages of foam cells in response to oxidative stress and inflammation, a process that ultimately contributes to the formation of the pro-thrombotic necrotic nucleus that characterizes mature atherosclerotic plaques [10]. Also,

macrophages housed at the site of the lesion contribute remodeling of the plaque, making it more prone to rupture by the production of proteases. Additionally, the presence and proliferation of autoimmune B cells within the diseased arterial wall in elderly mice with the atherosclerotic aortic disease has been observed [11]. Recent studies have shown that hypercholesterolemia considerably increases the number of circulating proinflammatory cells, thus accelerating atherosclerosis [10].

Based on this background, the contribution of nanotechnology to the health service is of particular attention. Specifically, the use of nanoparticles seems to represent an attractive therapeutic tool for the supply of anti-inflammatory drugs to specific cell types with the aim of attenuating or eliminating the immune actions in particular subgroups of cells, for example, restriction of myeloid cell differentiation and migration of proinflammatory monocytes to plaque [12]. In turn, the use of nanotechnology would make it possible to increase the bioavailability of poorly soluble drugs for the atherosclerosis treatment, which, when entering a cell matrix (due to its nanoscale), can dissolve and be absorbed more quickly. These advances would allow better management of the therapeutic regimen and the reduction of the frequency of administration of the mentioned drugs [13, 14].

2. NANOMEDICINE APPLIED TO ATHEROSCLEROSIS

Nanomedicine as an application of nanotechnology in the treatment, diagnosis, monitoring, and control of biological systems, is an unprecedented tool of medicine applied to pathologies, such as atherosclerosis and also allows overcoming the barriers of delivery of traditional pharmaceutical products. It is because the nanoformulated drugs can have a greater contact surface with the physiological medium where they are administered, which favors their dissolution being especially important for drugs with poor water solubility. This aspect, together with the amorphization suffered by certain crystalline drugs when they are encapsulated in nanostructures, considerably increases their bioavailability. Additionally, the

*Address correspondence to this author at the Departamento de Patología. Facultad de Ciencias Médicas. Universidad Nacional de Cuyo. Centro Universitario. CP 5500. Mendoza. Argentina; Tel: 54-261-4135000; Ext: 2739; Fax: 54-0261-4287370; E-mail: wmanucha@yahoo.com.ar

nanometric size of the aforementioned structures improves the passage through the biological membranes, contributing considerably to their absorption. Finally, the possibility of directing drug containment and release nanoplataforms to the target site of interest allows to reduce the adverse effects related to the systemic distribution of the drugs. At the same time, this feature maximizes their therapeutic efficacy using doses much lower than those that should be used to achieve the same effect with conventional pharmaceutical forms [15-17]. On the other hand, the biocompatibility and biodegradability inherent to the materials used in nanomedicine make this technology especially attractive for its use *in vivo* [8]. As described below, the most commonly used materials for the manufacture of nanoparticles in the biomedical field for the treatment and diagnosis of atherosclerosis have been organic polymers, such as collagen and polyesters, and metals and their oxides such as iron.

Of particular relevance are the studies carried out on macrophage cultures using phosphatidylserine-bearing nanostructures in combination with curcumin, an active ingredient with anti-inflammatory and antioxidant properties. These nanostructured lipid vehicles significantly inhibited the accumulation of lipids, the production of pro-inflammatory factors and also promoted the release of anti-inflammatory cytokines [18].

Also, a recent study carried out on atherosclerotic mice with nanoparticles of poly[lactic-*co*-glycolic acid-*b*-poly(ethylene glycol)] loaded with annexin 1 (with anti-inflammatory properties). Here, more than 70% of the nanoparticles reached the most advanced atherosclerotic lesions and verified their potential use in the treatment of chronic atherosclerosis. In this sense, a considerable improvement in the properties of the plaque was observed that included the suppression of oxidative stress, the increase in the production of collagen favors the protection of the plaque associated with a decrease in the activity of collagenase, and reduction of necrosis. Due to this, it was postulated that nanoparticles could act as agents that carry active ingredients with specific anti-inflammatory action and thus stabilize atherosclerotic lesions [19].

Similarly, the administration of collagen nanoparticles directed against the atherosclerotic plaque reduced the area of the lesion within the plaque, the necrotic nucleus, and oxidative stress [8].

In addition, systemic administration of acetylsalicylic acid decreases endothelial dysfunction, potentially reducing the formation of thrombus, the inflammatory infiltrate in the lesions, and the levels of steatosis and serum cholesterol [20], also improving vasodilation and inhibiting the progression of atherosclerosis. However, these results are minimized by a large number of side effects, such as cases of gastrointestinal bleeding in patients who are receiving this therapy; which justified the proposal to evaluate a hybrid stent with biodegradable nanofibers for the local and sustained release of acetylsalicylic acid in the damaged arterial walls. The experimental results suggest that biodegradable nanofibers release high concentrations of acetylsalicylic acid for approximately three weeks. Therefore, the proposed hybrid stent with biodegradable nanofibers of poly[lactic-*co*-glycolic] acid (PLGA) loaded with acetylsalicylic acid would contribute substantially to the sustained local delivery of drugs to promote re-endothelization and reduce thrombogenicity in the injured artery [21]. Although drug-eluting stents have been used in clinical practice for several years, the use of nanotechnology in the manufacture of them presents considerable advantages since it allows the treatment of lesions more extensive and complex than those commonly treated by traditional stents. This fact would decrease the possibility of restenosis or early clinical failures as a result of the use of these biomedical devices. Moreover, the use of nanotechnology makes possible to alter the texture of the stent surface or to design different types of coatings to improve endothelization and cell adhesion, reducing thrombosis. Furthermore, current designs of nanotechnology-based stents may contain more drugs and release them more specifically towards the vascular wall than the first generation of drug-eluting stents. Nanotechnology has al-

lowed introducing new materials and structures to the conventional stents that have provided essential improvements in them, reducing their disadvantages or limitations [22, 23].

Another study made similar contributions but through the development of a dual biodegradable stent of PLGA and poly-L-lactide loaded with two drugs of sequential and sustained release. One of these drugs was acetylsalicylic acid, with the purpose of taking advantage of its antiplatelet effect, and the other was paclitaxel used as an inhibitor of the proliferation of smooth muscle cells with the aim of attenuating the progress of vascular lesions [24]. Nanofibers induce a shallow inflammatory reaction in vascular tissues and are completely absorbed in 4 weeks [21].

In recent times a new paradigm has been generated on the specific orientation of functional nanoparticles. By connecting antibodies, proteins, peptides or other ligands to its surface, a nanoparticle can be targeted to an individual or multiple receptors that are expressed on the surface of (or within) an atherosclerotic plaque. For example, vascular targeting can be achieved using nanoparticles that have been functionalized with ligands specific for adhesion molecules, such as VCAM1, selectins or integrins such as αVβ3, since these adhesion molecules are expressed on the activated luminal endothelium of the affected blood vessel. Once the nanoparticle is bound to the specific receptor, it can remain attached to it or be internalized by the cell in question. The use of functionalized nanoparticles also offers the advantage that it does not translate into an increase in the percentage of the administered dose of the nanoparticle that reaches the specific lesion. Additionally, it allows for a better distribution of the nanoparticle between pathological lesions, increasing their entry to the endothelial cells [25]. It should be noted that, in spite of the numerous ligands proposed for pharmacological targeting, until now no ligand has been accepted as truly specific to atherosclerotic plaques [26].

In addition to its proposed novel therapeutic use, nanoparticles can be combined with agents for diagnostic imaging (*i.e.*, fluorophores, chelated ions, metals, among others). The contrast agents loaded in nanoparticles seem to contribute progressively with the diagnosis of atherosclerosis since the formation of these specific molecular images provides advantages over traditional contrast imaging agents which presents undesirable effects including tissue non-specificity and systemic toxicity.[8] While through nanotechnology, a new generation of contrast agents has been created that has overcome many of these challenges, and are able to provide more sensitive and specific information. Nanotechnology offers the possibility to adjust the chemical and physical properties of contrast materials to overcome the problems mentioned above as well as the valuable imaging time and signal strength. Moreover, nanotechnology allows precise control of particle size and surface properties that make possible to know the pharmacokinetic profiles of these new contrast agents. Additionally, by incorporating targeting techniques it is feasible to improve the precision of the imaging techniques, allowing for earlier diagnosis and treatment of lethal diseases. Moreover, nanomaterials allow combining many different materials at once, creating multimodal contrast agents capable of offering simultaneous structural and functional information about the human body [27].

The development of non-invasive techniques for monitoring and diagnosis of atherosclerotic plaques would allow more accurate visualization of them. An example of interest would be magnetic resonance imaging, which emerges as a promising non-invasive method for imaging atherosclerotic lesions, and the vessel wall of the artery with excellent spatial resolution [9].

The most studied class of magnetic nanoparticles for the development of contrast agents is the iron oxide nanoparticle, also known as the ferrite nanoparticle. This nanoparticle exhibits superparamagnetic, biodegradable, low toxicity nature, and its reactive

surface that can be modified with various biocompatible coatings [28].

3. CAUTION NOTES FOR NANOMEDICINE IN THE ATHEROSCLEROSIS TREATMENT

In the same way that the potential benefits of nanotechnological applications are considered in atherosclerotic disease, some disadvantages should also be taken into account. In this sense, the use of nanotechnology can be detrimental specifically concerning the design of bio-resorbable grafts to be implanted to restore the function of arteries damaged by atherosclerosis. These grafts are susceptible to calcification during the remodeling process, which implies a potentially fatal long-term complication [29].

On the other hand, the administration of some types of nanoparticles in healthy animals significantly deteriorates the vasodilator responses in the coronary arterioles and the mesenteric microvascular function. Moreover, *in vitro*, iron oxide nanoparticles induced cytoplasmic vacuolization, mitochondrial swelling, and death of human aortic endothelial cells; as well as, stimulation of the production of nitric oxide and increase of the adherence of monocytes. These results would indicate that exposure to nanoparticles represents a risk for the acquisition of atherosclerotic disease, and could also worsen the situation of patients suffering from this type of condition. Also, the intravenous injection of carbon nanotubes (MWCNTs) in rats fed a diet rich in lipids and vitamin D3, led to the development of atherosclerosis aggravated by a greater aortic lesion characterized by calcification. The interruption of tight endothelial junctions after exposure to MWCNTs indicated that increased turbulence and impaired endothelial function could trigger atherosclerosis. Consequently, even though nanoparticles are promising tools as vehicles for administering drugs aimed at treating atherosclerosis, particular attention must also be paid to their potential adverse effects [30]. It is thus relevant to have more information on the toxicity and biokinetics of the nanoproducts used for medicinal purposes and allow us to determine more precisely the real risks on the use of nanomaterials [31].

Also, although most of the polymers used in the field of nanomedicine are generally biocompatible, it is critical to making a careful evaluation of the interactions that are established between the nanoparticles and the components of the immune system, since many of these polymeric materials are immunogenic due to their exogenous nature. Therefore, we can begin to understand better the fundamental principles that allow the rational design of safe and useful nanomaterials [32].

CONCLUSIONS AND PROSPECTS

It is imperative to get a profound knowledge on prevention, diagnosis, and treatment of atherosclerotic disease, since it can be triggered not only in the absence of healthy habits and physical activity but also as a consequence of other types of non-traditional factors related to the immune system.

Nanotechnology and pharmacology are two branches of knowledge that can be used both individually and jointly to design new therapeutic alternatives that more comprehensively cover the analysis of multifactorial and lethal diseases, such as atherosclerosis. In this sense, the contributions to conventional treatments include the use of nanoparticles carrying anti-inflammatory drugs, the design of new kinds of stents for the delivery drug, and targeted pharmacological vectorization with specific molecules. Likewise, improving the diagnosis of the disease by combining nanostructures with fluorophores or other substances, poses an exciting and highly applicable field of study. Finally, the disadvantages and possible harmful effects that these methodologies may provoke must also be considered.

Summarizing, despite all the promising results mentioned in the field of research, there are still important challenges to overcome in clinical practice. Some of them are mainly related to the efficient and specific pharmacological vectorization, and potential nanotoxicity associated with the nanostructures used in the treatment of atherosclerosis.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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