



## Discussion

## Drug-loaded particles: “Trojan horses” in the therapy of atherosclerosis

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The Trojan Horse (In Greek: Δούρειος Ίππος, *Doúreios Híppos*; Fig. 1) is a tale from the Homer's Iliad about the subterfuge that the Greeks used to enter the city of Troy and win the war. After a fruitless 10-year siege, the Greeks constructed a huge wooden horse designed by Ulysses, and hid a select force of men inside. The Greeks pretended to sail away, and the Trojans pulled the horse into their city as a victory trophy ignoring the Trojan priest Laocoön who suspected that some menace was hidden in the horse, and warned the Trojans not to accept the gift, crying: “Beware of Greeks bearing gifts (In Greek: Φοβοῦ τοὺς Δαναοὺς καὶ δῶρα φέροντας)”. That night the Greek force crept out of the horse and opened the gates for the rest of the Greek army, which had sailed back under cover of night. The Greeks entered and destroyed the city of Troy, decisively ending the war.

Homer, Iliad [1].

Atherosclerotic disease remains one of the leading causes of death in the modern world [2]. The initiating pathophysiologic mechanism of atherosclerosis includes the subintimal accumulation of lipids, mainly low-density lipoproteins (LDL) [3]. LDL plays a

well-described pathological role in atherosclerosis. It is phagocytized by intimal macrophages, and in that process promotes the progression and eventual rupture of the atherosclerotic plaque. High-density lipoproteins (HDL), on the other hand, may play a protective role [4] as principal scavengers of cholesterol. However, while studies consistently demonstrate clinical benefits to LDL-lowering, clinical trials have thus far failed to identify a benefit to therapeutically increasing HDL concentrations. Nonetheless trials evaluating the clinical impact of HDL therapies are still ongoing, along with efforts to identify additional roles for that particle.

In concert with studies seeking new compounds against atherosclerosis, a number of innovative approaches to selectively deliver drugs to diseased tissues (including atherosclerotic plaques) have emerged. For instance, non-viral carriers based on polymer and lipid backbone have been used to deliver gene silencing molecules to cancer cells [5]. Other strategies include synthetic peptides that share common structural and biological features with native lipoproteins, thereby mimicking the endogenous natural mechanisms of an organism and potentially producing a beneficial effect [6].

Furthermore, several non-invasive imaging modalities have been developed that improve the assessment of atherosclerotic plaque morphology and metabolic activity. Magnetic resonance (MRI) allows the assessment of plaque inflammation in medium- and large-size arteries [7]. Positron emission tomography (PET) can also detect molecular processes, such as inflammation, *in vivo* [8,9]. While one of the main limitations of the radiotracers currently used clinically is that their uptake is not specific, new chemical engineering techniques are emerging and provide novel and sophisticated tracers that can overcome this barrier. Technological advances, such as those provided by combined PET/MRI cameras, provide additional innovations to the field of atherosclerotic plaques imaging.

In the context of these technological innovations, Zheng et al. [10] report (in this issue of *Atherosclerosis*) the use of HDL-mimetic peptide (CER-001) to target human carotid atherosclerotic plaques. CER-001 is an engineered lipoprotein complex consisted of recombinant human apoA-I and phospholipids, mimicking natural pre-beta HDL. The authors constructed a CER-001 molecule radiolabeled with Zr. Subsequently, its functional integrity was demonstrated *in vitro* in macrophages and *in vivo* in mice, and its stability

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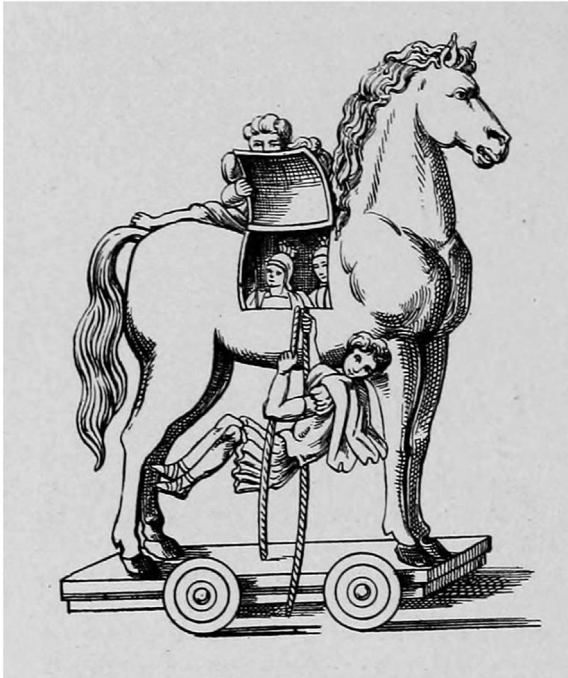


Fig. 1. Trojan horse.

in human blood samples was confirmed by gel electrophoresis. Thereafter, the authors sought to evaluate whether the radiolabeled CER-001 molecule selectively accumulated in human atherosclerotic plaques after IV administration. To do so, the HDL mimetic complex was administered to patients with >50% carotid artery stenosis and localization of radiotracer throughout the carotid wall

was examined using PET imaging.

The authors showed that the HDL mimetic particle is well tolerated by patients. Moreover, the authors report that radiotracer accumulated in atherosclerotic segments (those manifesting greater thickening in the carotid wall by MRI) and that radiotracer activity associated positively with the contrast enhanced MRI signal. Together, the study provides initial human data to suggest that the HDL mimetic could serve as a vehicle to more selectively deliver drugs and tracers to atherosclerotic plaques. Accordingly, the study illuminates a novel use for HDL, one that takes advantage of its predilection for lipid-rich lesions, which is less directly dependent on cholesterol scavenging and participation in reverse cholesterol transport.

Several limitations of the study are worth mentioning. Importantly, the clinical portion of the study had a very small sample size; studies in larger patient cohorts are warranted to better define the value of employing HDL mimetics to selectively deliver compounds to atherosclerotic plaque. Another important limitation was the lack of a control arm of healthy volunteers. While the study indicated a difference between more-vs. less-thickened locations in the carotid wall, it does not tell us about relative accumulation in non-diseased arteries. Furthermore, the absolute differences in tracer accumulation between more vs. less atherosclerotic segments was modest. Perhaps additional engineering may be needed to enhance the selectivity of the vehicle's targeting properties. Notwithstanding those limitations, the provocative findings of the study should prompt further investigations into this field.

To date, the systemic administration of statins has been one of the few strategies proven to be effective against atherosclerotic diseases [11]. The endeavor to provide more targeted treatments approaches aims to expand that armamentarium. In principle, targeted drug delivery represents an attractive approach, as it may enhance tissue effects, while minimizing systemic toxicity. Despite these theoretical advantages, targeted drug delivery has not thus

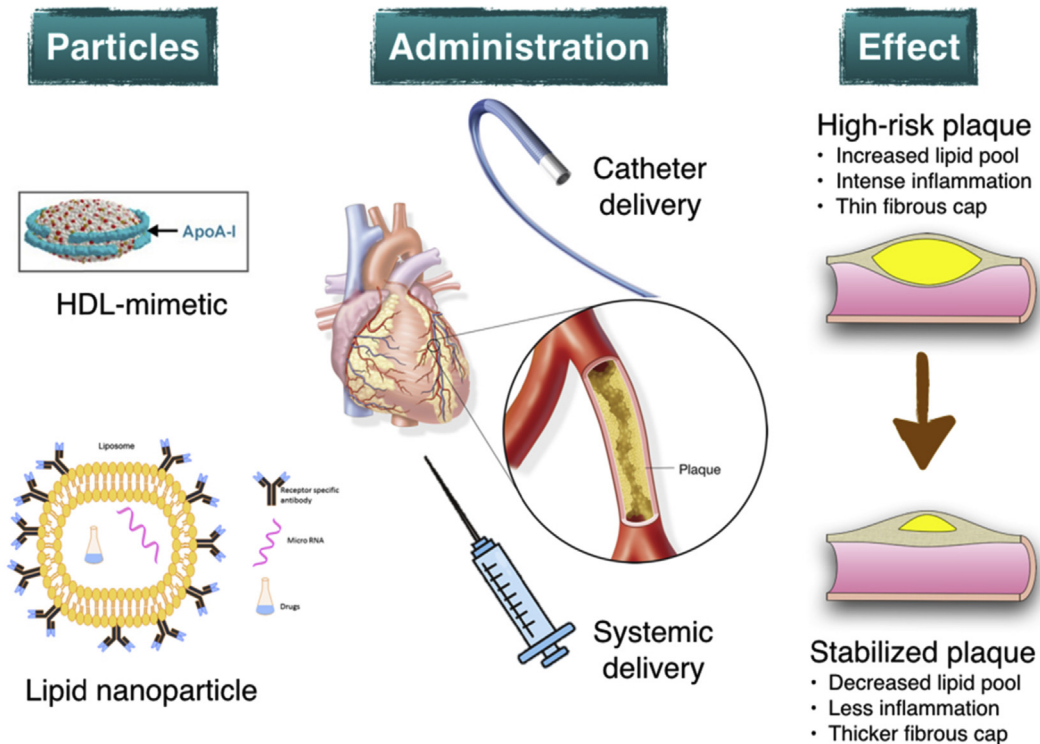


Fig. 2. Conceptual scheme of targeted drug delivery in atherosclerosis.

far been successful. Potential barriers to successful implementation of that strategy previously included: (i) lack of effective vehicles to deliver the drugs into the plaque, and (ii) inadequacy of diagnostic methods to assess and discriminate plaques at high risk for rupture from the more abundant vulnerable plaques. However, advances in imaging now facilitate the identification of high-risk plaques features [12,13], while breakthroughs in chemical engineering, nanotechnology and molecular biology enable the fabrication of small particles feasible for targeted drug delivery to humans [14]. These new molecules include peptide mimetics, as well as lipid nanoparticles (Fig. 2). The lipid nanoparticles can be administered systemically or locally via catheters (Fig. 2). They cross-link with specialized ligands against endothelial cell receptors to provide targeting capability to the plaque, where - like Trojan horses - they can release their cargo (i.e. drugs, microRNAs, etc), potentially reducing inflammation, reducing lipid core size and ultimately stabilizing the plaque (Fig. 2) [15,16]. Nanoparticle targeted delivery of drugs offers a great spectrum of advantages compared to systemic drug delivery. Reduced dose of drug, side effects and cost of therapy and increased accumulation of drug within the target tissue and patient compliance are the most prominent features of nanoparticle-mediated drug delivery [14].

Furthermore, targeted delivery of nanoparticles can be used not only for the treatment of atherosclerotic plaque, but also for potential diagnosis of high-risk atherosclerotic plaques. The concurrent use of cutting-edge multi-modality imaging cameras together with the application of molecular imaging agents may illuminate pathophysiologic and morphologic features of high-risk plaques in a manner that could open new diagnostic opportunities [8,17,18].

Efforts are underway to apply targeted probes to better identify and treat atherosclerosis, and to forge towards a new era of targeted medicine. The combination of novel imaging modalities in conjunction with nanoscale medicine has the potential to stimulate a shift towards this new era.

### Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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