Review

Searching for a successful HDL-based treatment strategy

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ABSTRACT

Despite strong evidence that HDL-cholesterol levels predict atherosclerotic events in a population, attempts at using an HDL-based treatment strategy have not yet been successful. Most of the efforts to date have focused on raising plasma HDL-cholesterol levels. This brief review focuses on a different strategy, which is based on the use of 18-amino acid apoA-I mimetic peptides. The story of these peptides spans decades and illustrates the remarkable complexity of HDL-based treatment strategies, but suggests that such a strategy may still be successful.

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1. Introduction

The predictive value of HDL-cholesterol levels for coronary heart disease (CHD) was established nearly a half a century ago [1]. Based on all the work done since then, we surely would have expected by now to have a therapy that raises HDL-cholesterol levels and reduces the risk for CHD. Unfortunately, this has not been the case despite multiple attempts to raise HDL-cholesterol levels by several pharmacologic approaches [2–6]. The results of these studies together with recent genetic studies [7] have caused some to question the value of raising HDL-cholesterol levels independent of changing LDL-cholesterol levels [8,9]. This brief review will take the view point that it may be irrelevant to ask if pharmacologically raising HDL-cholesterol levels will be a successful treatment strategy. The study of HDL has already led to a variety of novel approaches to the diagnosis and treatment of diseases,

which maybe successful independent of their ability to raise HDL-cholesterol levels [10–12]. This brief review will focus on attempts to use 18-amino acid HDL mimetic peptides for the treatment of disease. By focusing on the story of these 18-amino acid HDL mimetic peptides, we hope to illustrate some of the unexpected findings that have resulted from pursuing an HDL-based strategy. We believe that these findings are likely to lead to new therapeutic approaches regardless of whether raising HDL-cholesterol levels independent of LDL levels will be found to be an efficacious strategy.

1.1. Development of 18 amino acid HDL mimetic peptides for the treatment of atherosclerosis and other diseases with a chronic inflammatory component

The main protein in HDL, apolipoprotein A-I (apoA-I) has 243 amino acids. Searching for small peptides that do not have sequence homology to apoA-I, but which form a class A amphipathic helix and bind lipids similarly to apoA-I led to the discovery of an 18-amino acid peptide known as 18A. When the terminal charges of this peptide were modified by adding an acetyl group $(Ac-)$ and an amide group $(-NH_2)$ to produce $Ac\text{-}18\text{-}NH_2$ the affinity of the peptide for lipids increased. With the addition of these end blocking groups the peptide also became known as "2F" (Table 1). The name 2F was used because the peptide had 2 phenylalanine residues at positions 6 and 18 on the hydrophobic face of the class A amphipathic helix [13,14].

While the 2F peptide was effective in binding non-oxidized lipids, unfortunately it was not efficacious in vivo in a mouse model of diet-induced atherosclerosis [15]. A cell-based assay that measures

Table 1
Peptides discussed in this review.

2F	The peptide Ac-D-W-L-K-A-F-Y-D-K-V-A-E-K-L-K-E-A-F-NH$_2$
4F	The peptide Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH$_2$
5F	The peptide Ac-D-W-L-K-A-F-Y-D-K-V-F-E-K-F-K-E-F-F-NH$_2$
6F	The peptide Ac-D-W-L-K-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH$_2$
7F	The peptide Ac-D-W-F-K-A-F-Y-D-K-F-F-E-K-F-K-E-F-F-NH$_2$
6F without end blocking groups	D-W-L-K-A-F-Y-D-K-F-F-E-K-F-K-E-F-F

monocyte-chemoattractant protein-1 (MCP-1) activity generated by human artery wall cells after exposure to LDL was used to screen a panel of 18-amino acid apoA-I mimetic peptides [15]. Three peptides (4F, 5F and 6F) were found to be equally efficacious in decreasing LDL-induced MCP-1 activity in the cell-based assay, while 2F was only marginally effective. These peptides have 4, 5, or 6 phenylalanine residues, respectively, on the hydrophobic face of the class A amphipathic helix [15]. Interestingly, 7F with 7 phenylalanine residues was not effective [15].

The peptides 4F and 5F were subsequently found to be efficacious in a large number of animal models, virtually all of which had a chronic inflammatory component [16–18]. While full length human apoA-I and these 18 amino acid mimetic peptides bound non-oxidized lipids with similar affinity, the mimetic peptides were still able to bind the lipids with extraordinary affinity after they were oxidized; in contrast, full-length human apoA-I did not possess the ability to bind the oxidized lipids with high affinity [19]. When administered by injection, these peptides appeared to be equally efficacious whether synthesized from all D-amino acids or from all L-amino acids. This was felt to be due to the remarkable ability of the mimetic peptides to sequester the oxidized lipids in a fluid phase that was always tightly associated with the peptides in an aqueous environment [20,21]. Once tightly sequestered in the microenvironment of the peptide, the oxidized lipids lost their ability to interact with cells and hence lost their ability to initiate an inflammatory response [21].

1.2. Development of 18 amino acid HDL mimetic peptides for the treatment of cancer

In a search for biomarkers of ovarian cancer, it was discovered that HDL associated proteins including apoA-I, transthyretin and transferrin were predictors of early ovarian cancer [22–25]. Lysophosphatidic acid (LPA) has been implicated in cancer both as a possible biomarker and as a stimulator of growth and metastasis [26–29]. Interestingly, apoA-I was found to bind LPA with an affinity (K_D) of approximately 1 μM while the peptide 4F bound LPA with more than a 1000-fold higher affinity [30]. Using a mouse model of ovarian cancer in which the mice have normal immune function [31–33], Su et al. [30] found that transgenic expression of human apoA-I decreased tumor burden and significantly extended survival. Additionally, both the 4F and 5F peptides in vitro and in vivo (given either orally or by injection) decreased tumor growth and decreased supernatant and plasma levels of LPA in vitro and in vivo, respectively [30].

The mechanism of tumor inhibition by the apoA-I mimetic peptides was shown in part to be due to decreased tumor angiogenesis [34]. The inhibition of tumor angiogenesis was found to be due to peptide-mediated i) decreased Vascular Endothelial Growth Factor (VEGF) levels; ii) decreased activation of VEGF Receptor 2 (VEGFR2); iii) decreased activation of Fibroblast Growth Factor Receptor 1 (FGFR1); and iv) decreased downstream signaling molecules from these pathways [34]. Both human and mouse ovarian cancer cells were shown to produce VEGF in vitro and addition of LPA stimulated VEGF production. Adding L-5F together with LPA inhibited LPA-induced VEGF production, but pretreatment with L-5F did not, suggesting that LPA action requires binding of LPA to L-5F [34] consistent with known properties of these mimetic peptides [21].

It was also found that the apoA-I mimetic peptide 4F inhibited proliferation and tumorigenicity of epithelial ovarian cancer cells by upregulating the antioxidant enzyme manganese superoxide dismutase (MnSOD) [35]. Mouse ovarian epithelial cancer cells treated to inhibit the expression of MnSOD lost their responsiveness to treatment with the peptide suggesting that the induction of this antioxidant enzyme is part of the mechanism by which the peptide inhibits ovarian epithelial cancer cell proliferation [35].

HDL-cholesterol levels have been found to inversely correlate with risk for endometrial cancer [36] and HDL-cholesterol and apoA-I levels have been found to inversely correlate with risk for colon cancer [37]. In a mouse model of colon cancer, treatment with the 4F peptide administered in mouse chow decreased tumor burden, tumor angiogenesis and plasma LPA levels [38]. Additionally treatment with the 4F peptide administered in mouse chow reduced the number and size of tumors in the intestinal tract and decreased plasma LPA levels in C57BL/6J-Apc$^{Min/+}$ mice, a mouse model of familial adenomatous polyposis [38].

LPA was shown to induce hypoxia-inducible factor-1α (HIF-1α) in human ovarian cancer cell lines and in vitro treatment with the 4F peptide reduced the LPA-induced increase in HIF-1α [39]. Additionally, 4F treatment dramatically decreased HIF-1α expression in mouse ovarian tumor tissues [39].

The role of inflammation [40] including tumor associated macrophages [41] is well established in the progression and metastasis of tumors. Neyen et al. [42] demonstrated in vitro and in vivo that expression of scavenger receptor A (SR-A) on macrophages was necessary and sufficient to promote tumor invasiveness, and it did not seem to require additional signaling via toll-like receptor (TLR) pathways. Having previously shown that the 4F peptide was a potent inhibitor of SR-A [43], they demonstrated in vitro and in vivo that administration of the 4F peptide inhibited tumor invasiveness [42,44]. Interestingly, LPA is known to increase the expression of SR-A on macrophages [45].

2. Clinical trials of the 4F peptide

The first clinical trial of the 4F peptide was conducted with peptide synthesized from all D-amino acids administered orally in doses from 0.43 to 7.14 mg/kg [46]. Very low plasma levels were achieved; the highest dose produced a maximum plasma concentration of ~16 ng/mL. Despite the low plasma levels of peptide achieved, doses of 4.3 and 7.14 mg/kg significantly improved a measure of HDL functionality, the HDL-inflammatory index; doses of 0.43 and 1.43 mg/kg were ineffective [46].

The 4F peptide was designed to mimic a plasma protein (apoA-I) and it was extensively studied for its ability to improve the function of a plasma lipoprotein (HDL) [17]. Consequently, it was assumed that the critical therapeutic factor would be the concentration of peptide achieved in the plasma. Therefore, the next clinical trial was designed to achieve high plasma levels of peptide by administering low doses of the peptide by subcutaneous injection (SQ) or by intravenous infusion (IV) [47]. Doses of 0.042–1.43 mg/kg were tested even though these doses were ineffective in the first clinical trial. By administering the peptide SQ or IV, high levels of peptide were achieved with these low peptide doses (~3250 ng/mL). Surprisingly, despite these very high plasma levels, there was no improvement in the HDL-inflammatory index [47].

2.1. Understanding the failure of the second clinical trial

Returning to studies in mice, it was unexpectedly found that when equal doses of the 4F peptide were administered, the resulting level of peptide in the tissue of the small intestine was similar whether the peptide was administered orally or it was administered SQ [48]. In contrast, the levels in the liver or plasma after SQ administration were