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The Effect of Mimetic Peptide 4F on Paraoxonase-1

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short report

The Effect of Mimetic Peptide 4F on Paraoxonase-1

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Abstract

Even though there have been many advances in the diagnosis and treatment of coronary artery disease (CAD), CAD remains to be the major cause of deaths in the U.S. In humans, CAD is inversely related to levels of high density lipoprotein (HDL) cholesterol. The "quality" of HDL is just as important as the HDL levels. The major component of HDL, apolipoprotein (apo) A-I, appears to be largely responsible for the atheroprotective qualities of HDL. Apo A-I has been postulated to possess eight α-helical sequences. The majority of these sequences form class A structures that can be mimicked by several 18-residue peptide analogues. One such peptide, peptide 4F, has been found to inhibit atherosclerosis in atherosclerosis-susceptible mouse models. Also, peptide 4F increases paraxonase-1 (PON-1) activity in HDL in mouse models. PON-1 is an enzyme to which many of the anti-oxidative properties of HDL have been credited. The purpose of this study is to determine the effect of mimetic peptide 4F on PON-1 and provide insight into the mechanism by which peptide 4F effects PON-1. To examine the effects of 4F on PON-1, apo E null mice were treated with peptide L-4F and plasma and livers were harvested. The expected result was an increase in PON-1 activity in the plasma; however, preliminary results were not as expected and further experiments must be done to establish a conclusion.

Introduction

In spite of the advancement of treatments for coronary artery disease, the mechanisms in which the drugs prevent atherosclerosis are still unknown. It has been established that high levels of low density lipoproteins (LDL) and low levels of high density lipoproteins (HDL) contribute significantly to the development and progression of cardiovascular diseases (Parthasarathy, 2008). High density lipoprotein (HDL) is seen as one of the most important protective factors against atherosclerosis. Apolipoprotein (apo) A-I, the major component of HDL, is also inversely associated with coronary artery disease (Wilson, 1988). The protein apo A-I consists of 234 amino acids forming eight α-helical sequences that form class A structures. The manufacture of apo A-I is difficult and expensive, and therefore, research has been directed towards finding smaller peptide mimetics that produce similar results to apo A-I but are easier to manufacture and administer.

Peptide 4F was not homologous to the amino acid sequence in apo A-I but provided similar secondary structure, and also

contained 4 phenylalanine (F) residues on the hydrophobic face (Datta, 2001; Navab, 2005). Compared to apo A-I, it only consisted of 18 amino acids instead of 234 amino acids and 4 phenylalanine groups that provided high biological activity (Figure 1).

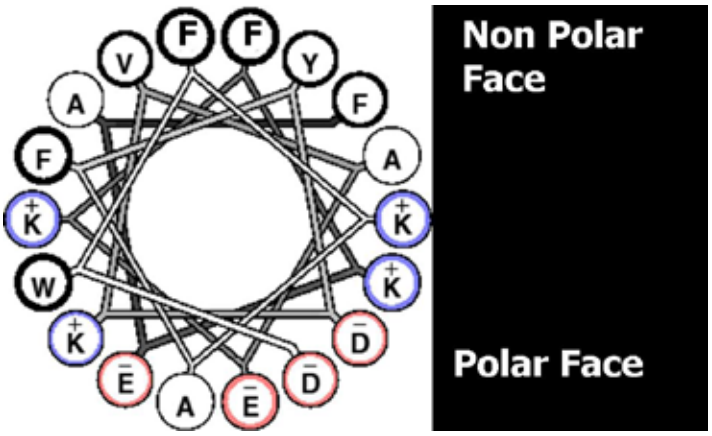


Figure 1: The Class A structure of peptide 4F

Paraoxonase-1 (PON-1) is an enzyme synthesized in the liver as an integral membrane protein (Bradshaw, 2005) and is secreted into the blood stream as a lipid vesicle precursor of nascent HDL (Oda, 2001; Deakin, 2002). Once in the plasma, PON-1 binds to apo A-I and is then found on the HDL complex (Figure 2). Studies have also shown that PON-1 is reduced in atherosclerosis and cardiovascular disease models and patients. Many antioxidative qualities of HDL have also been ascribed to PON-1. Previous research has shown that PON-1 destroys lipid hydroperoxides (LOOH), degrades oxidized LDL phospholipids, reduces accumulation of oxidized lipids in LDL, hydrolyzes oxidized LDL associated compounds, and inhibits both LDL and HDL oxidation (Florentin, 2008).

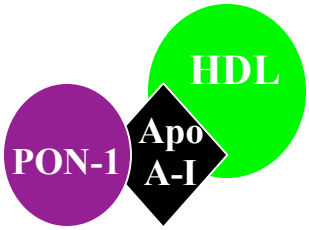


Figure 2: HDL complex with paraoxonase-1

After discovering the increase of PON-1 activity as a result of administration of peptide 4F, questions began to arise on the mechanism by which peptide 4F affected PON-1. Increased levels of PON-1 in the plasma can result either from increased synthesis of the enzyme in the liver or increased acceptor sites in the plasma. In order to increase the synthesis of PON-1, the peptide must affect mRNA levels by interacting with elements associated to the PON-1 promoter. These interactions would affect PON-1 expression and could therefore be hypothesized as a mechanism for 4F modulation. We hypothesize that 4F affects PON-1 by directly causing apo A-I to bind to PON-1 in nascent pre-β HDL and increasing PON-1 activity or by enhancing the genetic expression of the enzyme (or both) (Figure 3).

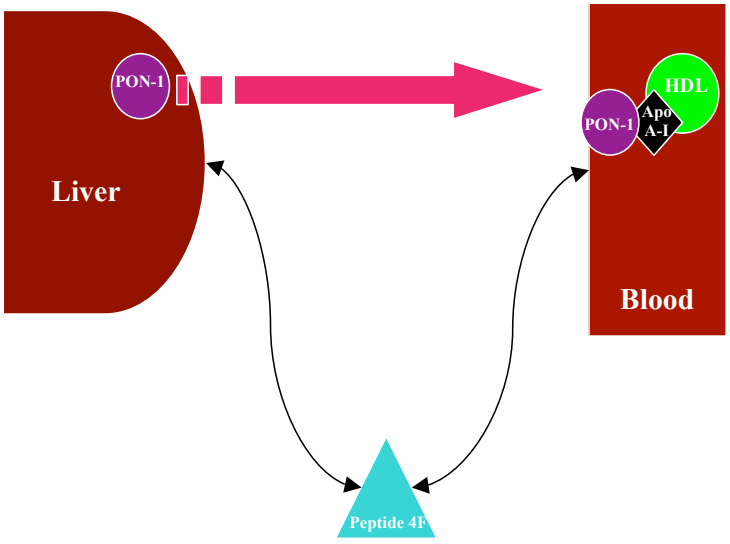


Figure 3: Hypothesized mechanisms of the effect of peptide 4F on PON-1

Methods

Subjects

30 six week old female apoE null mice were purchased from Jackson laboratories (Bar Harbor, ME). These mice were fed standard mouse chow diet (Ralston Purina). All mice were housed 3 per cage in autoclaved, filter-top cages under 12:12 hr light/dark cycle. Food and water was available ad libitum. All procedures were performed in accordance with the University of Alabama at Birmingham Institutional Animal Care and Use Committee with national regulations and policies.

Peptide synthesis

Peptide L-4F (i.e. Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH2) was synthesized by solid phase method using an automated solid phase synthesizer as previously described (Datta, 2000; Datta, 2001).

Injection Protocols

Peptide administration started at 7 weeks of age. Chronic administration of the peptide for 4 weeks would determine the chronic effects of the peptide. A baseline bleed was done when animals were 7 weeks of age to determine total plasma cholesterol levels. Animals were divided into 2 groups; n=15 per group. A

control group of 15 animals were injected with 200 µL of 0.9% saline everyday for 4 weeks. An experimental group of 15 animals were injected with 50 µg of L-4F (.5mg/ml concentration) i.p. everyday for 4 weeks. Mice were euthanized under xylazine/ketamine anesthesia for cardiac puncture blood collection and organ harvesting. Portions of livers were fixed in phosphate-buffered 4% formaldehyde for at least 24 h before freezing sections. Other portions were immediately placed in RNAlater (Qiagen) and stored at -80°. Blood was placed in heparinized microcentrifuge tubes and centrifuged (30min 12rpg) to separate the plasma.

Paraoxonase-1 activity

2 µL of whole plasma (for plasma PON-1 activity) was mixed with 200 µL of PON buffer (100 mmol/L Tris containing 2 mmol/L CaCl2, pH 8.0) containing paraoxon (1 mmol/L O,O-diethyl-O-p-nitrophenylphosphate) (Sigma) and the rate of formation of 4-nitrophenol over a period of twenty minutes was determined spectrophotometrically at 405nm. Blanks were included to correct for the spontaneous hydrolysis of paraoxon. The assay was performed in a 96-well plate (Costar) and readings were taken every 2 minutes.

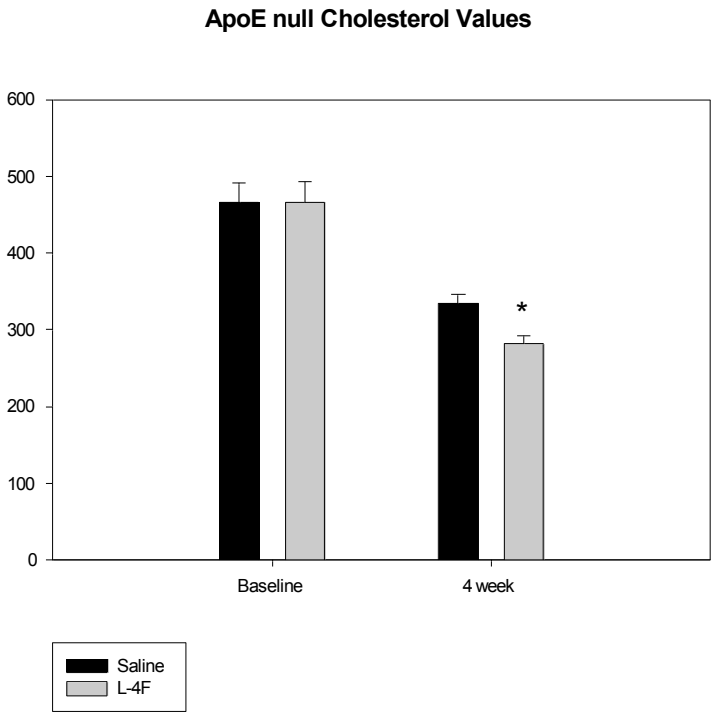
Cholesterol Levels

5 µl of standard/sample was mixed with 200 µL of Cholesterol reagent (cat # TR1342 from ThermoDNA, Arlington, TX) in a 96-well plate (Costar). The samples were incubated for 30min at room temperature. The absorbance was measured at 505nm vs. reagent blank.

Results and Discussion

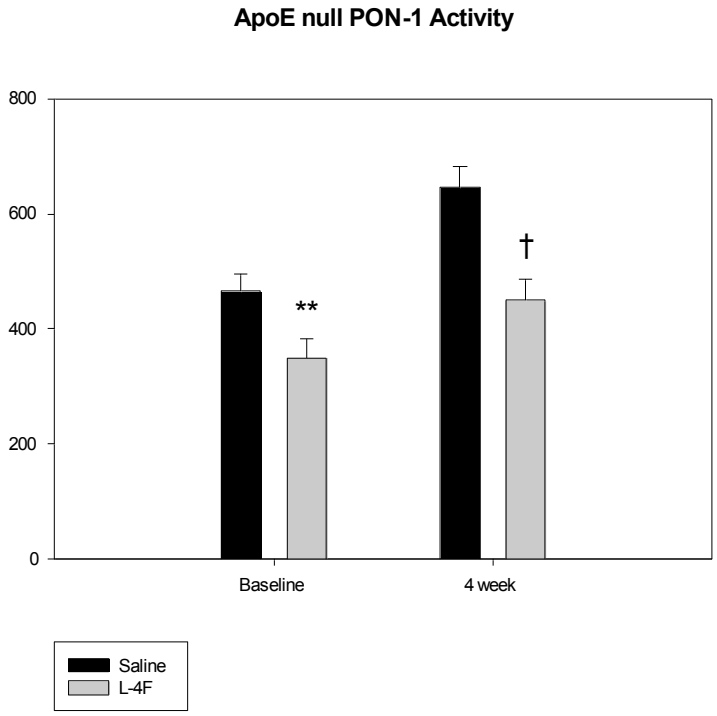
Administering 50µg of L-4F for 4 weeks significantly (*p<0.002) decreased cholesterol levels in the L-4F group compared to the control group (Figure 4). Previous studies have indicated that L-4F does not alter cholesterol levels. However, in the current experiment there was a decrease in cholesterol levels in L-4F treated mice.

PON-1 activity increased in both the control and the L-4F groups, but there was significantly (**p<0.02; †p<0.001) greater PON-1 activity in the control group compared to the L-4F group at both baseline and final measurements (Figure 5). This significant difference between the control group and experimental group at the baseline makes the interpretation of the data difficult. However, PON-1 activity varies between subjects in the natural environment, making it difficult to standardize both groups. Previous studies have exhibited a greater increase in the L-4F groups than the control groups. The PON-1 activity experiment provided results contradictory to those previously reported (Datta, 2001; Navab, 2005). Future studies will be needed to determine the anomaly that caused these results and the amount of PON-1 activated.



Cholesterol Levels

*Figure 4: Apo E null cholesterol levels. apoE null mice were injected with 50µg of L-4F or saline (n=15 in each group) and plasma cholesterol levels were compared to standards of 75, 150, and 300. The mice were bled at the beginning of 4 weeks and after 4 weeks of treatments. Cholesterol assays display a significant decrease of cholesterol levels in L4F treated mice than in control mice. The results shown are Mean±SD. *p<0.002 vs Control.*



PON-1 activity

*Figure 5: (bottom of page 32) Apo E null PON-1 activity. apoE null mice were injected with 50µg of L-4F or saline (n=15 in each group) and plasma PON-1 activity was determined. The mice were bled at the beginning of 4 weeks and after 4 weeks of treatments. PON-1 assays show an increase in PON-1 activity during the 4-week period of treatment with the peptide L4F. The results shown are Mean±SD. **p<0.02; †p<0.001 vs control.*

Limited Results

Due to this being an ongoing study and unexpected results, there are limited results based on the methods proposed. However, the next step is to conduct immunohistochemistry and Real-Time-PCR on histological liver sections and begin experiments with a human hepatocyte cell line, HepG2. We hope to discover a peptide and mRNA interaction causing an increase in genetic expression of PON-1 gene and an increase of enzyme PON-1 levels within the liver. With the cell line, we expect to find an increase of PON-1 secretion into the media and also an increase in genetic expression of the PON-1 gene.

Conclusion

Peptide 4F affects PON-1 activity and levels but conclusive data is yet to be determined.

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