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Anti-atherosclerosis effect of different doses of CETP vaccine in rabbit model of atherosclerosis

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ABSTRACT

Aim: To evaluate atheroprotective effects of different doses of cholesteryl ester transfer protein (CETP) vaccine, three doses of Tetanus toxoid-CETP (TT-CETP) peptide including 10, 50 and 100/rabbit, termed FA10, FA50, FA100, respectively, were administered in rabbit model of atherosclerosis.
Methods: Animals were vaccinated subcutaneously (S.C.) with 100 μl of vaccine in presence of complete Freund's adjuvant (CFA) for the first administration. Rabbits were boosted 4 times at 3 weeks intervals with the same peptide dose formulated in incomplete Freund's adjuvant (IFA). Animals were fed with diet supplemented with 2% cholesterol from week 11 to week 19. Anti-TT-CETP specific antibody and CETP activity in sera were determined. Therapeutic response was examined by tracking plasma lipoprotein levels (HDL-C, LDL-C and total cholesterol), and pathologic observation of intima/media thickness at the site of aortic lesions.
Results: All TT-CETP vaccine doses generated strong anti TT-CETP antibody response. CETP activity reduced in rabbits vaccinated with FA100 ($P = 0.031$). FA100 showed significant increase in level of HDL-C rather than control group ($P = 0.006$). However, no significant reduction were found in atherosclerotic lesion when compared to control.
Conclusion: Inhibition of CETP activity and increased HDL-C were found with FA100, but the vaccine failed to prevent aortic lesion development in immunized rabbits when compared to control. Our result supports the hypothesis stated that CETP may not be an attractive therapeutic target for the prevention of cardiovascular disease.

1. Introduction

Atherosclerosis, which now recognized as a chronic inflammatory disease, is characterized by accumulation of lipid and fibrous materials in the endothelium of arteries [1]. It is well established that immune response triggers by accumulation and oxidation of LDL in the arterial wall is involved in some pathological processes of atherosclerosis in high risk patients [2–4]. Accumulating evidences have demonstrated that HDL-C level is inversely associated with the risk of Cardiovascular events [5].

HDL participates in reverse cholesterol transport (RCT) pathway and has anti-inflammatory properties including inhibition of endothelial cell to express adhesion molecules and chemokines. HDL also inhibits the ability of antigen-presenting cells (APCs) to stimulate T cells [6]. Therefore, HDL-C based therapies have been suggested as a potential goal for the development of new therapies [6,7]. Although, current drugs include statins, fibrates and niacin, increase HDL-C concentration, the exact position of these drugs in

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reducing cardiovascular disease is not clear yet [8,9]. Other investigational drugs targeting HDL-C metabolism, reverse cholesterol transport or cholesteryl ester transfer protein (CETP) inhibitors are now under developing and some of them are currently undertaking evaluation in clinical trials [8,10].

Recently, increasing HDL-C level via inhibition of CETP is suggested to be a new strategy for prevention of cardiovascular events in high risk-patient [9,11]. Several epidemiological studies have shown that hereditary deficiency of CETP is associated with elevated plasma HDL-C [12,13].

CETP is a glycoprotein that is secreted by liver and circulates in plasma. The protein plays an important role in metabolism of the plasma lipoproteins by transferring neutral lipids and phospholipids between lipoproteins. CETP contributes to lower plasma HDL-C by transferring cholesteryl ester out of HDL particles into apo B-containing lipoprotein (LDL and VLDL). This protein also mediates the net mass transfer of triglycerides (TG) from TG-rich VLDL to HDL [14]. In numerous animal studies it has been shown that the inhibition of CETP by monoclonal antibodies, small molecules, antisense oligonucleotides and vaccines has elevated HDL-C level [15,16].

Vaccine is an immunotherapeutic approach for inhibiting CETP activity. Vaccine acts by eliciting antibodies which binds to and neutralize the activity of CETP [17]. The TT-CETP peptide, is a dimmer synthetic peptide that was first designed by Rittershaus et al. as CETP vaccine [18]. The vaccine was composed of 461–476 residues of human CETP C-terminal as a B cell epitope linked to residues 830–845 of tetanus toxoid (TT) as a T helper cell epitope. Recently, 100 μg of CETP related vaccine has been developed and the results have showed that the vaccine efficiently elicited antibodies against CETP and reduced susceptibility to atherosclerosis in rabbit model of atherosclerosis [19].

In the present study, we have evaluated the atheroprotective effects of different doses of TT-CETP peptide, similar to the chemically synthesized peptide vaccine described by Rittershaus et al. [18], in rabbit model of atherosclerosis. To evaluate immune response induced by selected vaccine doses, anti-TT-CETP specific antibodies and plasma CETP activity were determined. Therapeutic responses were examined based on the change in plasma lipoprotein levels. We also evaluated the effect of selected dose of TT-CETP on fatty-streak lesion development in rabbits.

2. Materials and methods

2.1. Materials

New Zealand White rabbits were obtained from Pastour Institute of Tehran (Tehran, Iran). TT-CETP Peptide were ordered from Peptrone (Peptrone, Korea) and was demonstrated to be >95% pure by high- performance liquid chromatography (HPLC) analysis. The amino acid sequence of peptide was as follows: CQYIKANSK-FIGITE-FGFPEHLLVDFLQSLS-amide. Complete Freund's adjutants (CFA) and incomplete Freund's adjuvant (IFA) were purchased from Sigma-Aldrich. All other materials were provided from other commercial sources with the highest purity availabled.

2.2. Animals

Rabbits were maintained individually in animal house of Pharmaceutical Research Center of BuAli Research Institute, Mashhad, Iran. Animals were kept under 12/12 h light/dark cycle at a temperature controlled (20–24 °C) with free access to food (standard laboratory diet, Javane Khorasan Co., Mashhad, Iran) and water. All animal experiments were carried out according to Mashhad University of Medical Sciences, Ethical Committee Acts.

2.3. Vaccination and atherosclerosis model

New Zealand White rabbits, weighing approximately 2 kg, were divided into three groups (n = 3–4). The average of serum lipoprotein levels and body weights were not significantly different between the groups. Animals were vaccinated subcutaneously (S.C.) with 100 μl of vaccine delivering 10, 50, 100 μg/rabbit TT-CETP peptide. The peptide were dissolved in sterile normal saline and emulsified (1:1 v/v) with CFA [20]. Control group were inoculated with normal saline. Rabbits were boosted S.C. 4 times at 3 weeks intervals with the same dose of TT-CETP peptide formulated with IFA. Since week 11, rabbits were placed on a diet supplemented with 2% cholesterol for inducing atherosclerotic lesions and maintained on this high cholesterol diet for 8 weeks. Blood samples were collected from fasted rabbits at weeks 0, 5, 8, 11, 15, and 19 and centrifuged (1500*g*, 15 min, 4 °C) to seprate serum. Sera kept frozen at −20 °C until being used. At the end of study, rabbits were euthanized and aortas were removed for atherosclerotic lesions analysis.

2.4. Anti-TT-CETP antibody analysis by ELISA

Anti-TT-CETP serum IgG was determined by ELISA at several time points as described before [21]. Bound antibodies were detected using peroxidase-conjugated goat anti-rabbit immunoglobulin (Abcam, USA) and 3,3′,5,5′-tetramethyl benzidine (TMB) substrate and the absorbance was measured at 450 nm. End-point antibody titers were determined based on the highest dilution of serum sample that gives twice times the mean absorbance obtained from control [22].

2.5. CETP activity assay

CETP activity in the serum of rabbits was measured with commercially available fluorimetric kit (Abcam, USA) according to manufacturer's instructions.

2.6. Lipoprotein analysis

To evaluate the effect of vaccine on serum lipoprotein level of vaccinated rabbits, the concentration of total cholesterol (total-C), HDL-C and LDL-C in sera of fasted rabbits were determined. Lipoprotein level were measured using routine commercial biochemical test kits with the enzymatic method (Biosystems, Spain). Atherogenic index was calculated using routinely used equations i.e. LDL/HDL.

2.7. Rabbit's aorta processing and atherosclerotic lesions analysis

At the end of the study (week 19), rabbits were euthanized by overdose of anaesthesia using Ketamin/Xylazin/Acepromazine, according to the guidelines [23]. Aortic arch were harvested and fixed in 10% formalin. The aortic arch was embedded in optimum cut temperature medium and sectioned. Sections were stained with hematoxylin and eosin (H&E). The extent of atherosclerotic lesions were analyzed by histological determination of the intima to medial thickness ratio assessed by two expert histopathologist in a blinded manner using a microscope (Olympus, Japan) equipped with a 10 Achroplan objective. The atherosclerotic thickness was assessed on an arbitrary scale 1–4 [24], as follows; *Trace*: Minimal thickening of subintima with little injury to arterial endothelium. *Grade1*: Plaque less than half as thick as the media with some form of endothelial dysfunction; increases in permeability to plasma constituents, including lipids; evidence of adherence of blood components (macrophages and platelets) to endothelium; macrophages and isolated foam cells inside the endothelium. *Grade2*: Plaque at least half as thick as media with