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Impact of evolocumab treatment on low-density lipoprotein cholesterol levels in heterozygous familial hypercholesterolemic patients withdrawing from regular apheresis

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ABSTRACT

Background and aims: Low-density lipoprotein (LDL) apheresis has been used to treat refractory hyperlipidemia such as familial hypercholesterolemia (FH). Evolocumab, a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor used in clinical settings, can reduce LDL cholesterol (LDL-C) levels by >70%. Therefore, this study aimed to assess the impact of evolocumab on withdrawal from regular LDL apheresis in patients with heterozygous FH (HeFH).
Methods: Eleven patients with HeFH undergoing biweekly LDL apheresis were enrolled and were subsequently switched to a biweekly subcutaneous injection of 140 mg of evolocumab. The primary endpoints were percent changes in mean LDL-C and apolipoprotein B (apoB) serum levels, which were averages of two different time point measurements, due to the switch in the treatment method.
Results: The mean LDL-C and apoB serum levels significantly reduced from 2.55 ± 0.62 mmol/L to 0.96 ± 0.40 mmol/L (−62.5%, $p < 0.0001$) and from 82.8 ± 12.3 mg/dL to 45.4 ± 10.9 mg/dL (−45.2%, $p < 0.0001$), respectively. Serum lipoprotein (a) levels also significantly reduced from 148 (116–351) mg/L to 91 (53–289) mg/L (−38.5%, $p < 0.01$). The reduction in LDL-C and apoB levels was not associated with the basal serum levels of PCSK9 or cholesterol production/absorption markers. Although evolocumab significantly reduced serum vitamin E levels, they were still within the normal range, and no subjective or objective side effects were observed.
Conclusions: Compared to biweekly LDL apheresis, biweekly evolocumab injection therapy is less expensive, less invasive, less time-consuming, and more effective in reducing atherogenic lipoprotein levels without severe adverse side effects.

1. Introduction

Familial hypercholesterolemia (FH) characterized by premature coronary artery disease (CAD) is the most severe form of monogenic hyperlipidemia [1]. Without effective cholesterol-lowering therapy, the onset of coronary atherosclerosis is estimated to be 22.9 years for men and 33.5 years for women [2]. Although statins can effectively reduce the low-density lipoprotein (LDL) cholesterol (LDL-C) level, FH is still refractory to conventional cholesterol-lowering therapy [3–5]. Although the co-administration of high-dose statin, ezetimibe, and cholesterol-sequestering resin can reduce LDL-C levels by 66% in heterozygous FH (HeFH) [5], the secondary prevention of CAD in FH is

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challenging.

LDL apheresis can mechanically and selectively remove apolipoprotein B (apoB)-containing lipoproteins, such as very low-density lipoproteins, intermediate-density lipoproteins, LDLs, and lipoprotein (a) [Lp(a)] from circulation [6]; however, LDL apheresis is an expensive, invasive, and time-consuming procedure. Moreover, LDL apheresis needs weekly or biweekly repetition so that LDL-C levels quickly return to baseline levels within 2 weeks after a single session of LDL apheresis [7].

Evolocumab was the first monoclonal anti-proprotein convertase subtilisin/kexin type 9 (PCSK9) antibody available for use in clinical settings, and it is expected to reduce LDL-C levels by > 70% [8]. Moreover, combined evolocumab and intensive statin therapy reduce cardiovascular events in patients with atherosclerotic cardiovascular disease [9]. However, inadequate data exist regarding the impact of evolocumab on LDL-C levels and other biomarkers after withdrawal from LDL apheresis in HeFH. Therefore, the objective of this study was to assess the efficacy and safety of evolocumab to enable withdrawal from regular LDL apheresis in Japanese patients with HeFH concomitantly treated with conventional oral lipid-lowering therapy.

2. Patients and methods

2.1. Study design

This was a prospective, nonrandomized, tri-center study in Japanese patients with HeFH, treated with biweekly LDL apheresis using the LIPOSORBER LA-15S (Kaneka Medix, Tokyo, Japan) system and concomitant stable pharmacological lipid-lowering therapy for more than 8 weeks. The study was registered at the University Hospital Medical Information Network (UMIN) (UMIN ID: 000022604 https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000026029). This study complied with the Declaration of Helsinki and was approved by the Ethical Committees of Kanazawa University Hospital, KKR Hokuriku Hospital, and Komatsu Municipal Hospital. Written informed consent to participate in the present study was obtained from each participant before starting the study.

The diagnostic criteria for HeFH were based on the Japanese Atherosclerosis Society Guidelines 2012, when two of the following three conditions were met: 1) primary hyper-LDL cholesterolemia (≥180 mg/dL); 2) existence of tendon xanthoma or nodular xanthoma on the skin; and 3) family history, within second-degree relatives of FH or premature CAD (for males <55 years and female < 65 years) [10]. No participants met our exclusion criteria: patients 1) with acute coronary syndrome within 8 weeks, 2) with anemia (hemoglobin concentration <10 g/dL), 3) under immunosuppressive therapy, 4) with severe hepatic disease (aspartate transaminase or alanine transaminase levels > 100 IU/L), 5) with severe renal disease (blood urea nitrogen level >14.3 mmol/L or creatinine level > 176.8 μmol/L), 6) who were pregnant or may become pregnant, and 7) who were allergic to evolocumab.

The efficacy and safety of LDL apheresis were assessed twice on weeks −2 and 0; subsequently, the patients were switched from biweekly LDL apheresis to biweekly subcutaneous injection therapy with 140 mg of evolocumab on week 0, the efficacy and safety of which were assessed twice on weeks 2 and 4. The primary endpoints were the percentage changes of mean LDL-C and apoB serum levels between LDL apheresis and evolocumab therapy. The average apoB, apoB-containing lipoprotein cholesterol levels, and fat soluble vitamin E during LDL apheresis were calculated as follows: $C_{average} = C_{min} + 0.73\ (C_{max} - C_{min})$, where $C_{average}$ is the mean concentration during biweekly LDL apheresis and C_{max} and C_{min} are the respective concentrations immediately before and after a single session of LDL apheresis [7,11] (Supplementary Fig. 1). The mean values of each biomarker during each therapeutic period were the averages of two different time points; the average value of weeks −2 and 0 for LDL apheresis period and the average value of weeks 2 and 4 for evolocumab injection therapy.

The secondary endpoints were the percentage changes in other lipid-associated biomarkers, such as total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, Lp(a), apolipoproteins, lathosterol, cholestanol, sitoseterol, and campesterol standardized by total cholesterol levels. The other biomarkers, such as PCSK9, fat soluble vitamins, and sex and adrenal hormones were also evaluated. Adverse events, subjective/objective symptoms, general laboratory test results (hematology, renal, and liver functions: skeletal muscle toxicity: and glucose metabolism) were observed as secondary endpoints.

2.2. Genetic diagnosis of FH

The method for the genetic diagnosis of FH is explained elsewhere [12,13]. Briefly, genomic DNA was isolated from peripheral white blood cells according to standard procedures, and polymerase chain reaction was performed. Primers covering all of exons and exon−intron boundary sequences of the LDL receptor and PCSK9 were designed using Primer3 online software (http://frodo.wi.mit.edu/). All 32 common, established LDL receptor gene mutations were screened in the patients, and LDL receptor gene mutations were ruled out using the following methods: the Invader assay method (Third Wave Technologies, Inc., Madison, WI, USA) for point mutations, the multiplex ligation-dependent probe amplification (MLPA) method for large rearrangements using a P062B LDLR MLPA kit (MRC Holland, Amsterdam, Netherlands), and the DNA sequencing method using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) for the other mutations.

2.3. Measurement of lipoproteins, lipids, and other biomarkers

Blood samples were collected immediately before and after LDL apheresis, and before evolocumab injection. The patients were not subjected to fasting to avoid hypovolemia during LDL apheresis. Blood samples were centrifuged immediately at 4 °C. Serum cholesterol and triglyceride levels were measured using enzymatic methods, and HDL-C levels were measured using the polyamine polymer/detergent method (Sekisui Medical, Tokyo, Japan). Serum apolipoprotein levels were measured using the immunoturbidimetry method (Sekisui Medical, Tokyo, Japan), and serum Lp(a) levels were measured using the latex enhanced immunoturbidimetry method (Nittobo Medical Co. Ltd, Tokyo, Japan). Serum levels of PCSK9 were measured as hetero-dimers using the sandwich enzyme-linked immunosorbent assay (BML, Kawagoe, Japan). The levels of serum plant sterols, sitosterol and campesterol, lathosterol, which is a precursors of cholesterol biosynthesis, and cholestanol, which is a metabolite of cholesterol, were measured by gas-chromatography (BML, Tokyo, Japan). The serum levels of fat soluble vitamins A and E were measured using high performance liquid chromatography (BML, Tokyo, Japan).

2.4. Statistical analysis

Values are expressed as mean ± SD or median (1st and 3rd quartiles). Differences in the levels of lipids, apolipoproteins, and other biomarkers between LDL apheresis and evolocumab therapy were compared by the paired *t*-test for parametric data and the Spearman's rank correlation coefficient for non-parametric data. The associations between percentage reductions in LDL-C or apoB