Treatment of Severe Hypercholesterolemia in the Prevention of Coronary Heart Disease

International Symposium 'Treatment of Severe Hypercholesterolemia in the Prevention of CHD', Naples, June 16 and 17, 1988

Treatment of Severe Hypercholesterolemia in the Prevention of Coronary Heart Disease

Volume Editors
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Preface

In June 1988 an international symposium on the 'Treatment of Severe Hypercholesterolemia in the Prevention of Coronary Heart Disease' was held in Naples, Italy.

Why was this symposium organized? First of all, we were impressed by the fact that, by removing low density lipoproteins (LDL) from the plasma of patients with severe hypercholesterolemia, we were finally able to observe what we had been striving towards for years: the disappearance of skin lesions in homozygous familial hypercholesterolemic patients: angina disappeared in many of them and they willingly accepted this therapy. This symposium provided us with an opportunity to discuss these important findings with many other colleagues, some of them experts in this field. Familial hypercholesterolemia had been a very interesting model for many years, during which we had been trying to understand its mechanisms. However, a turning point came with the discovery of LDL receptors by the Nobel Prize winners Goldstein and Brown, who shed new light on this problem and provided us with much fascinating new information. Even in therapy, there is an old saying in this field that one case of familial hypercholesterolemia, especially homozygous, is equal to ten or more common cases of hypercholesterolemia. In other words, if it is possible to reduce cholesterol in such extreme cases, then the compound used must be a very good one. Thus LDL-apheresis became a stimulating source of ideas on how difficult cases could be treated. Then it was just a short step from hypercholesterolemia itself to coronary patients, since this method was also able to improve the flux in many arterial areas, and in some cases also to reduce the size of atheromas.

This led us to the question: Could this method be applied not only in familial hypercholesterolemia, but also in coronary heart disease in general? The papers presented at this symposium represent an important step towards answering this question. Hopefully, this book will stimulate other researchers to pursue this topic further, perhaps leading to the establishment of new research programs in many centers.

We would like to thank all the authors who have contributed to this book, Dr. Agostino Gnasso for his care in collecting the manuscripts, as well as Karger Verlag for their efficiency in publishing the book.

Last but not least, we would like to express our sincere thanks to the Kanegafuchi Chemical Industry (Kaneka) for supporting this symposium. We are very grateful for the considerable effort they put into it and are confident they will be pleased at having aided such a worthwhile international scientific venture.

Naples, August 1988 The Editors

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The Lipid Hypothesis

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It was a great pleasure for me to co-chair this important conference with Professor Mancini. I will introduce the conference by summarizing the current evidence supporting the cholesterol connection to coronary heart disease.

The cholesterol or lipid hypothesis states that an increase in serum cholesterol, and in particular LDL cholesterol, will increase the risk of coronary heart disease (CHD), while a decrease in cholesterol will lower CHD risk. One cannot assume, however, the latter relationship from the former. While we know from the Framingham data that increased blood pressure is linked to increased CHD [1], none of the placebo-controlled hypertensive trials has shown that a reduction in blood pressure resulted in a decrease in coronary disease. Lowering blood pressure in these trials decreased cerebral vascular accidents, and thereby lowered overall mortality, but the decrease in coronary disease has never been more than 25% of that predicted from the Framingham data.

Nonetheless, overwhelming evidence supporting the cholesterol-CHD connection comes from a variety of sources including experiments with laboratory animals and epidemiologic studies between and within populations. For example, average cholesterol levels in Southern Italy are much lower than in Northern Europe or the United States, and the death rate from coronary disease in Southern Italy is correspondingly lower than in the other two regions [2]. Moreover, in parts of Japan and Asia, where average cholesterol levels are below 140 and 150 mg/dl, there is virtually no atherosclerosis or coronary disease despite the prevalence of smoking and hypertension [3].

Some of the most extensive epidemiologic data relating elevated cholesterol levels to increased CHD risk come from the Multiple Risk Factor Intervention Trial (MRFIT) [4]. Data from over 360,000 men screened for the MRFIT trials show a continuous relationship between cholesterol and CHD mortality beginning at cholesterol levels of approximately 180 mg/dl [5, 6]. This relationship is curvilinear, with an inflection in the curve between cholesterol values of approximately 220–240 mg/dl (fig. 1).

In contrast to the positive relationship of total and LDL cholesterol to CHD, a strongly inverse and independent relationship exists between HDL and CHD. The latest Framingham data show that the risk of myocardial infarction for female subjects increased approximately six-fold as their HDL levels decreased from 65 to 45 mg/dl [7]. Similar but less striking changes were seen in males.

Genetic studies of diseases, such as familial hypercholesterolemia and apo-A-I/C-III deficiency, also lend strong credence to the cholesterol-CHD connection. Individuals with heterozygous familial hypercholesterolemia have only half of the normal level of LDL receptors; thus, their cholesterol and LDL levels are increased, causing myocardial infarctions to occur prematurely [8]. Patients with the rare apo-A-I/C-III deficiency, in which HDL is essentially missing, often experience overwhelming atherosclerosis early in life [9].

Primary and secondary prevention trials using lipid-lowering drug therapies provide even further support for the cholesterol hypothesis. The

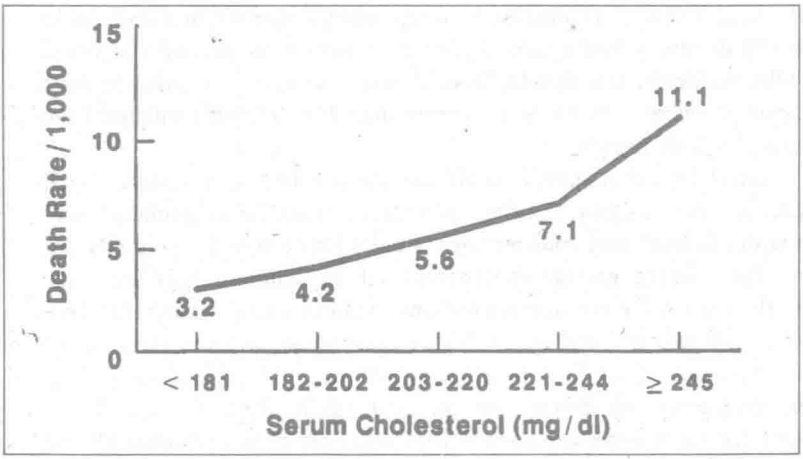

Fig. 1. Quintiles of serum cholesterol and 6-year death rate per 1,000 for 356,222 primary screenees in the MRFIT (men aged 35–57).

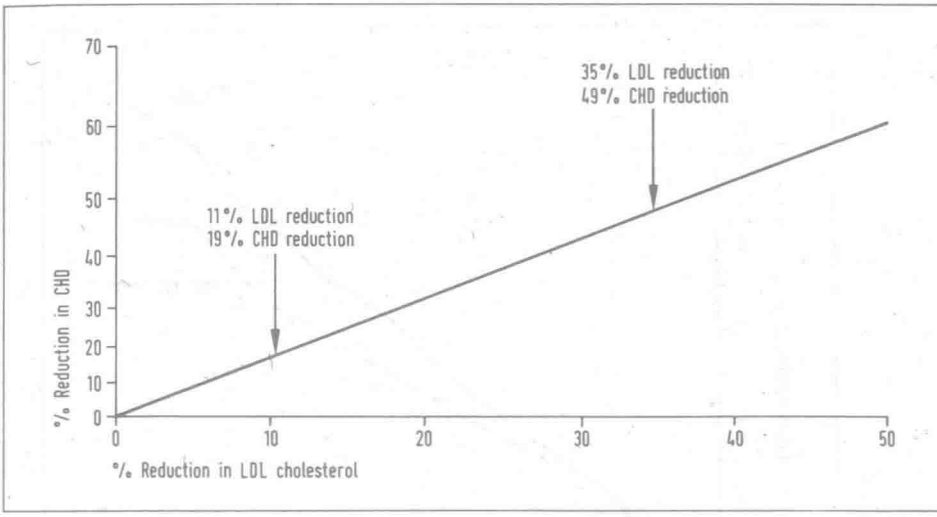

Fig. 2. Relationship between lowering of LDL cholesterol and reduction of CHD.

results of the Coronary Primary Prevention Trial [10] (CPPT) provided the most definitive evidence at that time linking lower cholesterol to lower CHD. The CPPT was a double-blind, placebo-controlled trial treating hypercholesterolemic men with diet and cholestyramine. The subjects, treated over a 7-year period in 12 North American clinics, experienced an 11 or 12% decrease in LDL cholesterol compared with a placebo group. This decrease in LDL cholesterol translated to a 19% decrease in CHD, measured as non-fatal myocardial infarction and/or CHD death. Furthermore, the CPPT results indicated a linear relationship between the degree of LDL cholesterol lowering and CHD reduction (fig. 2). Those individuals who were unable to take the drug had no reduction in CHD, while those who were able to take the full drug dose experienced a 20% reduction in cholesterol, a 35% decrease in LDL cholesterol, and an almost 50% decrease in coronary disease.

From this study, the so-called 1-to-2 formula was derived, namely, that a 1% reduction in cholesterol is associated with a 2% decrease in non-fatal myocardial infarction and/or CHD death.

More recently, the results of the Helsinki Heart Study [11], added another dimension to the cholesterol hypothesis. In this placebo-controlled, double-blind study, a similar group of asymptomatic men having high

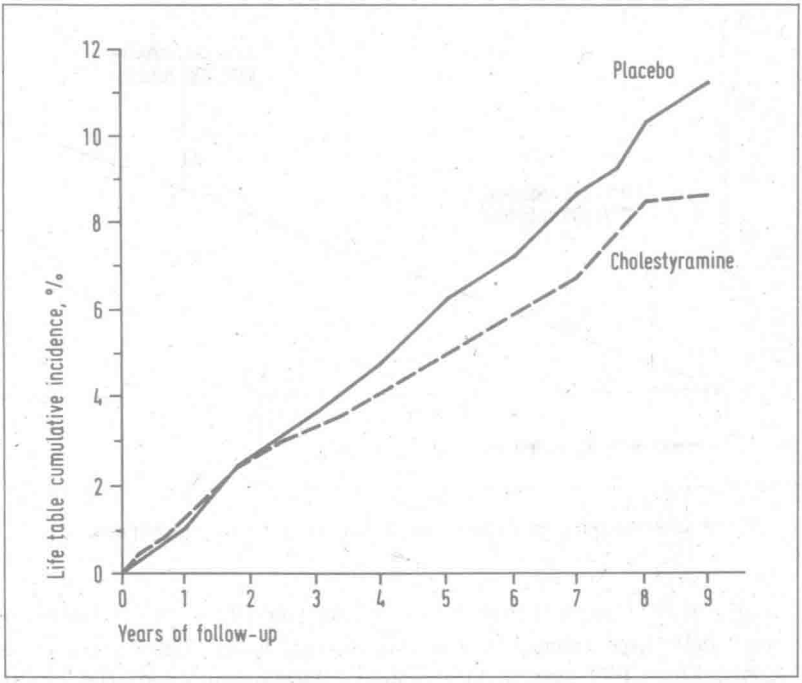

Fig. 3. Primary endpoint life table.

cholesterol levels were treated with gemfibrozil. About two-thirds of the patients had type IIa hyperlipidemia patterns at the trial's entry, while some 30% had type IIb, and about 10% type IV. As with the CPPT, curves indicating the endpoints of myocardial infarction and/or CHD death for the treatment and placebo groups did not diverge until after 2 years (fig. 3, 4). Moreover, when the endpoints of the Helsinki Heart Study were analyzed on a yearly basis, an accelerating effect of the treatment was noted after year 2, with CHD reduced by almost 50% in the gemfibrozil group in the third to fifth years of the study. Thus, the Helsinki and CPPT results suggest that about 2 years of lipid modification are required in order to see an effect on coronary events. After that point, as indicated by the Helsinki Heart Study, the effect appears to accelerate.

Over the entire 5-year Helsinki Heart Study, cholesterol and LDL cholesterol decreased by 8–10%. Based on the 1-to-2 formula of the CPPT, one would have expected the 8–10% drop in total cholesterol to lead

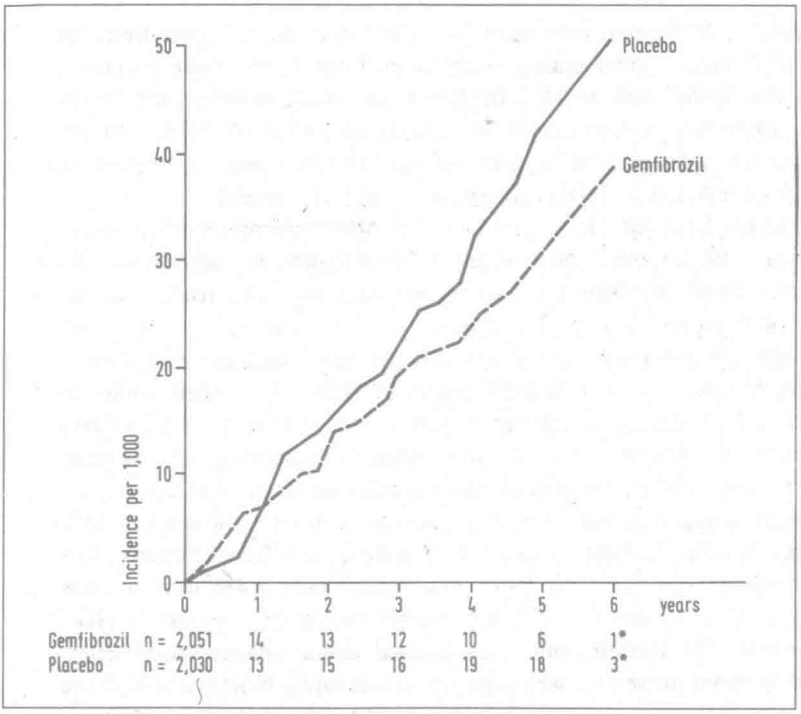

Fig. 4. Kaplan-Meier cumulative incidence (per 1,000) and annual number of cardiac endpoints, according to treatment group and time. Data for the sixth years (asterisks) were derived from 305 person-years of observation for gemfibrozil and from 316 person-years of observation for placebo.

to a CHD reduction of about 16–20%. Instead, there was a CHD reduction of 34% in the Helsinki treated group. The larger-than-expected decrease was attributed to the combined effects of raising HDL cholesterol and decreasing LDL cholesterol.

Thus, the Helsinki Heart Study provides the first evidence from a clinical trial that raising HDL, in conjunction with lowering total and LDL cholesterol levels, will cause a more substantial reduction in CHD than would be obtained just from lowering LDL cholesterol alone.

Is it possible to induce regression of coronary atherosclerosis and if so, what levels of LDL reduction, HDL increase, or combination therapy are required to achieve that effect? A study in Great Britain on plaque size [12] provides some of the first evidence that lipid reduction may retard lesion

progression of femoral atherosclerosis. Further evidence comes from the Type II Coronary Intervention Study carried out at the National Heart, Lung, and Blood Institute (NHLBI) [13]. In this study, patients who had the most progression of coronary disease had the lowest ratio of HDL cholesterol to total cholesterol, while those who had the least progression had the highest proportion of HDL cholesterol to total cholesterol.

The results of the Cholesterol Lowering Atherosclerosis Study (CLAS), reported in 1987, provide the strongest evidence to date that decreasing LDL cholesterol and/or raising HDL cholesterol may induce regression of coronary plaques in man [14]. The subjects in this study, all of whom had had coronary artery bypass surgery, were divided into treatment and placebo groups. Subjects in the treatment group, pre-selected for their ability to tolerate a high dosage of colestipol, were treated with diet, 29.5 g of colestipol and 4.5 g of nicotinic acid. In contrast, the placebo group was given dietary advice and a homeopathic dose of nicotinic acid. After 2 years, the treatment group experienced a 26% decrease in total cholesterol, a 43% decrease in LDL cholesterol, and a 37% increase in HDL cholesterol. The placebo group, on the other hand, experienced only a 4% drop in total cholesterol, a 5% drop in LDL cholesterol, and a 2% increase in HDL cholesterol. The investigators also obtained global change scores determined by quantitative coronary angiograms measuring blockage in both the native and the bypassed vessels. It is important to note that the investigators were not measuring changes in a single lesion but rather the overall state of obstruction of the coronary vessels. Using the global change scores, the investigators found that 16.2% of the treatment group versus 2.4% of the placebo group had evidence of coronary plaque regression.

A final question raised by critics of the cholesterol hypothesis is whether lipid modification affects mortality. Preliminary clues are provided by the Framingham data. Men aged 31–39 with cholesterol values averaging over 260 mg/dl had the least likelihood of surviving 30 years, while those with cholesterol levels averaging under 180 mg/dl had the greatest likelihood of surviving [7]. However, many years will be required before we have final answers on the mortality question.

In closing, let me give a brief perspective to the potentially beneficial role of LDL-apheresis. The first application of LDL-apheresis could be aimed at the small fraction of patients with severe hypercholesterolemia who are refractory to the usual dietary and drug treatment. A second and broader use could be in the treatment of CHD as a method of halting or regressing the development of coronary plaque. This latter application, of

course, is still highly speculative [15]. I believe that a clinical trial is in order to determine the degree of LDL-reduction achievable with apheresis and/or drug therapy which will, in turn, stop atherosclerosis and induce regression in susceptible lesions.

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Genetics of Familial Hypercholesterolemia

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Hypercholesterolemia is a major predisposing cause of coronary artery disease. In this chapter, I shall review the genetics of hereditary hypercholesterolemia. Classical familial hypercholesterolemia (cFH) is the result of inherited defects in the low density lipoproteins (LDL) receptor. However, recent evidence suggests that the underlying defect in familially transmitted hypercholesterolemia can also involve the ligand, i.e., the structure of apolipoprotein (apo) B-100.

Classical Familial Hypercholesterolemia

The studies of Brown and Goldstein [1] have provided a detailed account of the metabolic fate of LDL, the major carrier of serum cholesterol. Circulating LDL bind to cell surface receptors with high affinity, the liver being the major organ that takes up LDL by this pathway. The receptor-ligand complexes cluster in coated pits and become internalized by endocytosis. The internalized LDL are contained in vesicles that eventually fuse with lysosomes. Within the lysosomes, LDL apo B-100 is degraded completely to amino acids, and LDL cholesteryl esters are hydrolyzed to generate free cholesterol. The latter serves as a second messenger responsible for suppressing 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. In addition, LDL-derived cholesterol activates a cholesterol-esterifying enzyme, acyl CoA:cholesterol acyltransferase, so that excess cholesterol can be stored in the cytoplasm as cholesteryl ester droplets; it also suppresses the synthesis of LDL receptors by lowering the concentration of receptor messenger RNA (mRNA). The modulation of LDL recep-

tor by the LDL-derived cholesterol ensures that the appropriate amount of cholesterol is taken up by the cell to meet its metabolic needs without the risk of overaccumulation.

The LDL receptor contains 6 distinct structural domains [1–4]: (1) a signal sequence; (2) a ligand-binding domain, which mediates the interaction between the receptor and lipoproteins that contain apo B-100 or apo E; this domain is composed of 7 repeats of ~40 amino acids, each repeat containing 6 cysteine residues that probably form 3 intrarepeat disulfide bonds, resulting in a conformation that presents negatively charged amino acid residues on the surface of the receptor; (3) a domain homologous to the epidermal growth factor precursor, which may function as part of the ligand domain, or as a hinge region to remove any intramolecular steric hindrance; (4) a domain of clustered O-linked sugars rich in serine and threonine residues; (5) a transmembrane domain of 22–25 hydrophobic amino acids; and (6) a cytoplasmic domain that may be involved in targeting the LDL receptor to coated pits in the plasma membrane.

The human LDL receptor gene spans 45 kilobases (kb) of DNA on the distal short arm of chromosome 19. It contains 18 exons and 17 introns. There is a strong correlation between structural domains in the protein and exon sequences in the LDL receptor gene. Detailed accounts of the structure of the LDL receptor gene have been published [3, 4].

Classical FH (cFH) is the result of mutations in the LDL receptor gene that lead to the altered expression, structure and/or function of the LDL receptor. Characterization of homozygous FH fibroblasts reveals that mutations at the LDL receptor locus can disrupt any of the following processes: (1) synthesis; (2) transport; (3) binding; and (4) clustering and internalization of the LDL receptor. The most common class of mutations comprises mutant alleles that fail to produce an immunoprecipitable LDL receptor protein (null allele). This class has also been labeled crm^- (cross-reacting material minus) mutants. Hobbs et al. [9] analyzed the LDL receptor in 132 subjects with homozygous cFH. Sixteen of the 132 cFH cell strains synthesized no immunoreactive LDL receptor protein, and were thus homozygous for crm^- mutant genes. DNA and mRNA from 15 of the 16 crm^- patients, representing 30 crm^- genes, were studied. Haplotype analysis based on 10 restriction fragment length polymorphisms suggested that the 30 crm^- genes represent 13 mutant alleles. Four of the alleles produced no mRNA. Three of these 4 $mRNA^-$ alleles had large deletions ranging from 6 to 20 kb that eliminated the promoter region of the gene. The gene for the other $mRNA^-$ allele was grossly normal. Nine alleles were

mRNA$^+$ of which 3 encoded mRNAs of abnormal size. The other 6 alleles appeared normal by Southern blotting and produced normal-sized mRNA, but no immunoreactive receptor protein.

Mutations that affect subsequent steps in the LDL receptor pathway have been described [1, 3]. It is apparent that mutations of almost any type, including insertions, deletions, nonsense and missense mutations have been found. Therefore, in general, in an individual patient with cFH, the exact mutations may not be predictable. However, individuals within certain ethnic communities or geographic locations have been found to share common mutations. For example, a number of Lebanese cFH patients were found to have a nonsense mutation at amino acid 660 [10], and the majority of French Canadian cFH alleles were found to have a large deletion (>10 kb) in the gene for the LDL receptor [11].

The very elegant studies of Brown and Goldstein summarized above provide the molecular basis for cFH. It is evident that there is much heterogeneity in the genetic defects in cFH. However, they all involve the structure and expression of the LDL receptor and thus plasma cholesterol levels, since about 60–70% of LDL cholesterol is removed via the LDL receptor pathway. Heterozygous cFH has a frequency of approximately 1 in 500. Homozygotes occur about 1 in a million. cFH is therefore a relatively rare genetic disease, accounting for less than 5% of the patients admitted to coronary care units following a myocardial infarction. In the vast majority of patients, other genetic and environmental factors play important parts in the development of coronary artery disease. In the remainder of this chapter, I shall discuss the potential contribution of heritable defects in an LDL receptor ligand, apo B-100, to non-classical FH.

Non-Classical Familial Hypercholesterolemia: Potential Role of Heritable Defects in Apo B-100

Apo B-100 is the major protein component of LDL and is the major physiological ligand that is recognized by the LDL receptor. Apo B has been recognized as the major protein component of LDL for many years. However, apoprotein(s) with similar properties were also found in chylomicrons, very low density lipoproteins (VLDL), and intermediate density lipoproteins (IDL). The work of Kane et al. [12, 13] indicates that the apo Bs found in different human lipoprotein particles are heterogeneous: apo B-100 is present in LDL, IDL, and VLDL, whereas apo B-48 is present