Does Therapeutic Intervention Achieve Slowing of Progression or Bona Fide Regression of Atherosclerotic Lesions?

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Abstract—This review focuses on the regression of atherosclerosis in humans and experimental animals. It highlights the difficulties to determine unequivocally whether with a given therapeutic intervention, such as diet, drugs, or apheresis, the progression of lesions was curtailed or bona fide regression of atherosclerotic lesions was achieved. It seems appropriate to mention that 2 very different ways to measure regression were used in experimental animals and in humans. Regression in animals was determined mainly in the aorta or coronary arteries isolated at post mortem, and the criteria used were degree of sudanophilia and/or aortic wall thickness and cellular composition or cholesterol content. In humans, the evaluation of regression relied mainly on quantitative coronary angiography. The literature of the past decade is reviewed selectively but not exhaustively, and in some instances, a brief historical overview is given. (Arterioscler Thromb Vasc Biol. 2001;21:183-188.)

Key Words: atherosclerosis ■ prevention of CAD ■ fatty streaks ■ complex lesion ■ gene therapy

Atherosclerosis and coronary heart disease are still considered the major cause of death in Western society, but the availability of new diagnostic and therapeutic procedures have resulted in a significant reduction in mortality. It is therefore important to define whether these recent approaches have resulted in the slowing of progression or the bona fide regression of atherosclerotic lesions.

Definition of Atherosclerotic Lesions

The first, or early, lesion (type I) consists mainly of microscopically and chemically detectable lipid deposits in the intima.1 When the lipids are intracellular, mainly in macrophages, the lesion is designated as type II, or fatty streak. During the development of the atheroma, the fatty streak progresses to a more mature lesion and goes through several transformations. The more advanced lesion consists of layers of macrophage foam cells and lipid-laden smooth muscle cells. The next stage (type III lesions) contains in addition to the lipid-laden cells of type II, scattered collections of extracellular lipid droplets and particles that disrupt the coherence of intimal smooth muscle cells.1 Lesions of type IV also contain a large core of extracellular lipid, formed through the coalescence of scattered collections of extracellular lipid.1 Around the fourth decade of life, some of the lesions of type IV acquire a thick layer of connective tissue and are designated type V. When type V lesions develop a fissure, hematoma, or thrombus, they become type VI lesions.1 The study of the regression of such complex lesions in humans presented an immense technical and ethical hurdle.

Studies in Experimental Animals

Rabbits

To induce atherosclerosis in rabbits, the animals are usually fed diets that contain 1.0% to 1.5% cholesterol and saturated fat. To study the regression of existing lesions, the rabbits are switched back to the basal diet. It became apparent that on return to the normal diet, there was even progression of atherosclerosis, because during the induction, the liver became engorged with cholesterol that took many months to clear and continued to secrete atherogenic lipoproteins. Therefore, therapeutic approaches were initiated to enhance cholesterol removal from the aortic lesions by feeding cholestyramine, which increases the loss of body cholesterol.2 In that study, use was made of a nonhydrolyzable analog of cholesteryl ester (CE) (ie, 3H-cholesteryl linoleoyl ether [3H-CLE]), which after intravenous injection entered the aortic lesion and was retained there. During the 330 days of the regression period, there was no discernible loss of the 3H-CLE but there was a significant loss of CE, and thus, the change in the ratio of 3H-CLE to CE could serve as a quantitative marker of regression.2 Another regimen of enhanced regression consisted of administering fish oil and/or verapamil after 10 weeks of 0.3% cholesterol feeding; a significant reduction in aortic lesions as determined with planimetry of sudanophilic areas was described within the next 4 weeks.4 Badimon et al5 fed rabbits a cholesterol-rich diet for 60 days, injected an HDL-VHDL fraction (weekly) during an additional 30 days on the same diet, and reported significant regression of lesions. In a later study,6 rabbits fed cholesterol for 105 days were returned to the normal diet for...
60 additional days, during which they were injected weekly with apoA-I, the major apolipoprotein of HDL. The treatment resulted in lesser cholesterol and CE accumulation compared with nontreated control animals, but regression was not achieved. These seemingly contradictory results can be explained by the difference in experimental design. In the shorter experiment, predominantly fatty streaks were induced, which can regress more easily (vide infra). In the longer experiment, there was apparently a reduction in cholesterol content (ie, fatty streaks), but the more complex lesions did not regress. Repeated injections of phospholipid vesicles to cholesterol-fed rabbits resulted in significant cholesterol mobilization and regression of aortic intermediate lesions.

A different approach to the study of regression was the long-term administration of L-arginine, the precursor of NO, to rabbits fed a cholesterol-rich diet. The L-arginine induced an improvement in endothelium-dependent relaxation that was accompanied by a reduction in the surface area of sudanophilic lesions, indicating the regression of preexisting lesions. The authors attribute the effect of L-arginine on lesion regression to the induction of apoptosis in macrophages. The application of MRI technology to study atherosclerosis in rabbits was reported during the past few years and permitted the estimation of atherosclerosis in vivo. The study was performed in cholesterol-fed rabbits that underwent aortic balloon injury; the regression of aortic atherosclerosis was initiated by return to chow diet for 20 months. MRI examination after 4 months of regression showed 56% stenosis, but after 20 months, it decreased to 45%. Similarly, average plaque thickness decreased from 0.85 to 0.6 mm between 4 and 20 months of regression. The WHHL rabbit, an LDL-receptor–deficient model of atherosclerosis that develops aortic lesions on a chow diet, was used in the past decade to study the effects of drug intervention. Another attempt to enhance regression of atherosclerosis in cynomolgus monkeys was reported in a study designed to test the effect of pravastatin on plaque characteristics independent of cholesterol-lowering effect. A 2-year induction period was followed by a 2-year regression phase. The salient findings were that although pravastatin did not affect plaque size in coronary or iliac arteries, there were fewer macrophages in the intima and media of the pravastatin-treated monkeys, and a better dilator function of the arteries was noted.

**Monkeys**

Regression studies of diet-induced atherosclerosis in aorta and coronary arteries in nonhuman primates began with the pioneering work of Armstrong et al and were admirably reviewed by Wissler and Vesselinovitch. Of special significance is the conclusion that atherosclerosis in monkeys bears a remarkable resemblance to that found in humans. The results obtained showed that return to a low-fat diet for 12 to 14 months is accompanied by a 60% reduction in gross aortic intimal lesions, and the addition of choleystamine further reduces the amount of aortic surface involvement. These results were obtained in rhesus monkeys but not in cynomolgus monkeys, in which the regression of atherosclerosis was not quite so successful. One difference between the 2 strains of monkeys is the abundance of immune complexes in cynomolgus monkeys and the extension of plaques into the media; the latter feature is not seen in human atherosclerosis.

An important distinction was made by Strong et al that to study the regression of advanced lesions, the period of induction of atherosclerosis must be increased. In experiments that last for up to 9 years, the induction period was increased to 5.4 years and regression was increased to 3.7 years. Return to basal diet resulted in a significant decrease of fatty streaks in most arterial segments. After 1.9 years, the raised lesions were less numerous, but no regression was noted. After 3.7 years on the regression diet, there was a nonsignificant decrease in the raised lesions. In that study, in addition to morphometry, CE content of arterial segments was determined. There was a 50% reduction after 1.9 years and a 75% reduction in CE after 3.7 years on the regression diet. The authors made an important caveat that although fatty streaks may regress almost completely, “regression of atherosclerotic lesions does not induce reversion of the arteries to a prediseased normal state.”

These results just reviewed were derived mainly in male monkeys, but during the past decade, the investigations were extended to female monkeys and included the evaluation of hormonal therapy. It seems of interest that these studies were carried out on cynomolgus monkeys, which are apparently less prone to regression. The animals were ovariectomized and fed the atherogenic diet for 2 years. Thereafter, regression was induced by lipid-lowering diet either alone or in combination with hormonal replacement. After 30 months on the various regression regimens, the plaque size and the lumen in coronary arteries did not change from that in the progression group. On the other hand, cholesterol content of the aorta was reduced ~50% by the lipid-lowering diet, but there was no further improvement with hormonal therapy. These results showed that estrogen replacement with or without progesterin offered little or no improvement of regression in ovariectomized females. In an extension of that study, the effect of hormonal replacement on connective tissue was determined. Conjugated estrogen treatment inhibited collagen accumulation that was associated with atherosclerosis regression. Another attempt to enhance regression of atherosclerosis in cynomolgus monkeys was reported in a study designed to test the effect of pravastatin on plaque characteristics independent of cholesterol-lowering effect. A 2-year induction period was followed by a 2-year regression phase. The salient findings were that although pravastatin did not affect plaque size in coronary or iliac arteries, there were fewer macrophages in the intima and media of the pravastatin-treated monkeys, and a better dilator function of the arteries was noted.

**Swine**

Minipigs have been used for the study of atherogenesis for many years, and Cornhill et al were the first to introduce quantitative analysis with the help of computer-stored digital images of Sudan-IV–stained arteries. The further development of this methodology generated probability-of-occurrence maps that defined in statistical terms the probability that sudanophilia will occur at a given point along the surface of the arterial tree. This method permitted the morphometric analysis of the Pathological Determinants of Atherosclerosis in Youth (PDAY) Study (see later).

The feeding of an atherogenic diet to miniature swine for 6 months resulted in the development of fatty streaks in the thoracic aorta and fibrous plaques in the abdominal aorta. During the next 9 months of regression on a conventional
diet, serum lipid levels returned to baseline and there was regression of fatty streaks but not of fibrous plaques. The induction of atherosclerosis for 8 months, followed by a regression period of 4 months that included feeding of fish oil, did not induce the regression of atherosclerosis but prevented the progression of established lesions. In a later study, the effect of fish oil was examined in minipigs fed the atherogenic diet for 8 months followed by a normal diet alone or with fish oil. Although significant regression of lesions in the aorta and in carotid arteries occurred in animals on the regression diet, there was no potentiation of regression by the addition of fish oil.

Hamsters
Among small rodents, hamsters have been used in studies on atherogenesis because they respond to high-cholesterol diets with an increase in plasma cholesterol levels. However, when hamsters were fed 1% cholesterol for up to 20 months and their plasma cholesterol increased by >5-fold, the cholesterol content of the total aorta increased not more than 28% above that found in age-matched control hamsters. This relative resistance could be related to the commensurate 4-fold increase in plasma HDL levels. Significant regression of fatty streaks was reported in a study of 10 weeks of induction and 8 weeks of regression. The regression of fatty streaks was also found in another study with a low-fat, low-cholesterol diet with or without supplementation of lovastatin. In a recent study, the effect of coconut oil versus olive oil on regression of lesions in the ascending aorta and aortic arch was compared after 8 or 16 weeks. In the ascending aorta, coconut oil increased the extent of lesions, whereas with olive oil, no regression occurred up to 16 weeks. Different results were reported for the aortic arch, in which both coconut oil and especially olive oil enhanced regression.

Transgenic Mice
The introduction of transgenic technology to the study of atherosclerosis resulted in the creation of mice that responded to a Western diet with lesions that resemble those found in humans. The most commonly used models were either LDL receptor–deficient or apoE-deficient C57BL mice, in which overexpression of human apoA-I or apoA-IV resulted in significant prevention of the development of atherosclerosis. When regression was attempted by a reduction in plasma cholesterol levels with the feeding of phytosterol, this treatment slowed the development of atherosclerotic lesions in apoE-deficient mice but did not affect regression. In another model of mouse atherogenesis, namely the apoE-3-Leiden transgenic mice, withdrawal of the atherogenic diet fed for 14 weeks and feeding of a standard diet for up to 16 weeks resulted in normalization of plasma cholesterol levels after 4 weeks. At 4, 8, and 16 weeks on the standard diet, the size of the aortic plaques tended to get smaller, but the difference did not reach statistical significance. However, the percent of macrophages in the lesions was significantly reduced after 4 weeks and approached zero after 16 weeks. In another study, an apoE-expressing retrovirus was used to transduce apoE-deficient mouse bone marrow for transplantation into apoE−/− recipient mice. When mouse or human apoE was expressed in these mice between 5 to 13 weeks of age, a significant decrease in lesion area was seen. However, when macrophage apoE was expressed at a later age (10 to 26 weeks), there was no effect. The authors conclude that “macrophage apo E can delay atherogenesis if expressed during foam cell formation, but is not beneficial during the later stages of atherogenesis.” When human apoE expression in the liver of apoE−/− mice was achieved with the help of second-generation adenoviruses, marked regression of preexisting fatty streaks occurred in 12-week-old mice. The apoE was expressed as above in 26-week-old mice, it induced a more moderate regression of lesions during 6 weeks of the experimental period. During that time, there also was a significant decrease in plasma cholesterol levels. In a more complex study, apoE−/− mice were generated on the nude background as a new mouse model for atherosclerosis. Human apoE cDNA was transferred to these mice with a first-generation adenoviral vector. The nude athymic mice are deficient for cellular immunity, and because of their immunodeficient background, the adenovirus-mediated gene transfer and the expression of apoE lasted for >4 months. In these 17-week-old mice, a dose-dependent regression of fatty streak lesions occurred during 4 months after the gene transfer. In another model system, namely, LDL receptor–deficient mice, liver-directed gene transfer of apoA-I was performed. Plasma levels of human apoA-I peaked 7 days after injection and declined during the subsequent week. A remarkable regression of lesions in the aortic root was observed 4 weeks after the adenovirus injection. In the treated mice, the percent lesion area occupied by macrophages and macrophage-derived foam cells was significantly reduced.

Studies in Humans
Regression of Early Lesions
Atherogenesis is a multistep process with arrest possibilities at various stages. The fatty streak, universally acknowledged as the initial stage of atherogenesis, consists of the accumulation of lipid-laden macrophages and is designated type II lesion (Stary et al., Figure 5). In infants within the first year of life, fatty streaks were found in aorta and coronary arteries and appeared to be related to breast feeding. Between age 1 and 4 years of age, there was a marked reduction in these lesions, indicating regression at the very early stage. More recently, these findings were extended to human fetuses, and a correlation was found with maternal hypercholesterolemia during pregnancy. In addition, determination of the lesion area in the aortic arch revealed that the largest lesion per section was found in children up to 3 years of age and was significantly smaller than that in fetuses aged 6 months. These results supported the notion that fatty streaks may already regress during late pregnancy.

The natural history of atherosclerosis in childhood and young adulthood was studied extensively in a multicenter cooperative project. The study, PDAY, examined the relation of risk factors for adult coronary artery disease (CAD) in almost 3000 persons aged 15 to 34 years who died of external causes. The study provided strong evidence that progression of the early lesions was positively associated with plasma LDL and VLDL levels and negatively associated with HDL levels. Smoking was associated with a 3-fold increase in raised lesions in the abdominal aorta in the 25- to 30-year-old group. Even though fatty streaks develop in many areas of
abdominal aorta, only fatty streaks on the dorsolateral surface of distal aorta progress to become raised lesions.\textsuperscript{50} It seems plausible that the introduction of healthy lifestyle habits (diet low in fat and cholesterol, physical activity, balanced energy intake with expenditure, no smoking) in childhood could not only stop the progression but also promote the regression of lesions.\textsuperscript{46,48–50}

**Regression of Advanced Lesions**

As mentioned in the introduction, in contradistinction to studies in experimental animals that evaluated the regression of atherosclerosis in the aorta and the coronary, iliac, renal, femoral, and other arteries, human studies deal mainly with coronary and carotid arteries. With the use of quantitative coronary angiography (QCA), the regression of atherosclerosis in humans has been defined indirectly in terms of arteriographic improvement in stenosis, which is expressed as increase in luminal area in the stenotic vessel.\textsuperscript{51} The quantitative term $\Delta$ (% S) used to designate progression with a plus sign or regression with a minus sign denotes the average change in percent stenosis over all the lesions measured per patient. With the advent of effective lipid-lowering therapy, many randomized secondary prevention angiographic trials were initiated and provided information concerning the magnitude of regression. The regression trials of coronary atherosclerosis using different lipid-lowering drugs were summarized extensively,\textsuperscript{52,53} so the individual trials are not reviewed here. A summary of 7 trials showed an improvement in $\Delta$ (% S) that ranged from $-0.7\%$ to $-2.2\%$ in stenosis in the treated patients, whereas the $\Delta$ (% S) worsened from $+0.8\%$ to $+3.4\%$ of luminal area in controls.\textsuperscript{51} There are some caveats that have to be taken into account when using this method; improvement in lumen size can occur independent of changes in plaque size due to remodeling of the vascular wall\textsuperscript{54} or relaxation of the vascular tone.\textsuperscript{55,56} Spurious increase in the lumen may also result from lysis of ocluding or mural thrombi.

Therefore, notwithstanding the very marked clinical improvement and reduction in CAD mortality rates, as well as total mortality rates, with the effective lipid-lowering therapy, the extent of regression of lesions in the coronary arteries was disappointingly low. Hence, additional treatments were introduced to promote regression of lesions, especially in familial hypercholesterolemia (FH) patients.

**LDL Apheresis**

The term “LDL apheresis” was introduced by Stoffel et al\textsuperscript{57} to describe the selective removal of apoB-containing lipoproteins from plasma in vivo through immunoabsorption. LDL apheresis has been used mainly for the treatment of patients with FH or with very severe CAD and high plasma LDL cholesterol levels that did not respond adequately to dietary and drug intervention. In the first major multicenter study, after 2 years of treatment, stenosis of coronary arteries improved by 8% in one fourth of arterial segments.\textsuperscript{58} In a randomized study, FH heterozygotes with CAD received twice-weekly dextran-sulfate LDL apheresis plus simvastatin, or colestipol and simvastatin.\textsuperscript{59} QCA was performed in 39 patients before and after 2 years of treatment. Six patients were found to be progressors and 9 were found to be regressors, with $\Delta$ (% S) values of $-1.8\%$ on apheresis and $-2.2\%$ on drugs. In this cohort, no advantage of LDL apheresis was found.\textsuperscript{59} In another study,\textsuperscript{60} 42 men aged 30 to 67 years with CAD were treated with LDL apheresis (bi-weekly) and simvastatin or simvastatin alone. After 2 years of treatment, the mean plasma LDL cholesterol in the LDL apheresis group was reduced by 63\%, and that in the medication group was reduced by 47\%. Nine patients in the apheresis group and 11 in the medication group were classified as progressors, and 2 and 5 patients, respectively, were classified as regressors. In a prospective study,\textsuperscript{61} 25 FH heterozygotes were treated with LDL apheresis and drugs and 11 patients were treated with drugs only; the patients underwent QCA 2.5 years thereafter. The frequency of regression or no progression was significantly higher in the apheresis group. Another application of LDL apheresis was made in long-term heart transplant survivors with angiographically documented CAD and severe hypercholesterolemia.\textsuperscript{62} In this study, before the initiation of LDL apheresis, the luminal diameter of the coronary arteries decreased from $3.6\pm1.1$ to $3.15\pm1$ mm at 22 months after the heart transplantation. During the next 22 months of LDL apheresis, the luminal diameter increased to $3.4\pm1.5$ mm. This improvement could have been due to regression and improved vascular tone.\textsuperscript{62} The effectiveness of LDL apheresis was recently reviewed.\textsuperscript{63,64}

**Conclusions**

The results presented in the current review provide evidence that the induction of fatty streaks in humans occurs during the early prenatal phase and correlates with maternal plasma cholesterol levels; the regression of these fatty streaks can be very rapid even in utero. Fatty streaks also develop postnataally, possibly in conjunction with high lipid intake provided by milk. These fatty streaks are also easily regresisible and disappear after the third year of life. The PDAY Study established the correlation between the presence of fatty streaks in adolescence and risk factors for CAD (such as high non-HDL cholesterol, low HDL cholesterol, and smoking) and provided evidence that in certain locations, fatty streaks will progress to complex lesions. The development of fatty streaks in rabbits, monkeys, swine, hamsters, and mice is also achieved by the induction of hypercholesterolemia, usually through dietary regimens. The regression of fatty streaks after the normalization of plasma cholesterol levels is dependent on the rate of cholesterol efflux from macrophage foam cells and can be enhanced by an increase in reverse cholesterol transport.

The induction of advanced lesions in experimental animals is a much slower process than the induction of fatty streaks and requires prolonged exposure of the arterial wall to hypercholesterolemia. Attempts to regress these complex lesions were successful in the reduction of cholesterol content but to a much lesser extent of the nonlipid components of the lesions. The decrease in lesional cholesterol was related to the extent of lowering of plasma atherogenic lipoprotein levels and the duration of intervention.

In the evaluation of the results of treatment, it is important to distinguish between slowing of progression and true regression of lesions, which are 2 different processes dependent on different mechanisms.
It must be borne in mind that the current techniques to evaluate regression in humans are not optimal, because they do not allow differentiation of the lesions in vivo. Therefore, the knowledge accrued from the study of regression of advanced lesions in experimental animals is most relevant to the situation in humans. In long-term trials of secondary prevention of CAD, the increase in the partially obstructed lumen of coronary arteries, which is due in part to regression, was disappointingly small. Despite this, dietary and drug intervention did culminate in marked clinical improvement and reduction in CAD and total mortality rates in about one third of patients. These results were due in part to removal of cholesterol from the lesion, resulting in plaque stabilization, and in part to improvement in the vasomotor tone. It is expected that when a more aggressive lowering of LDL cholesterol to levels prevalent in primates will be the goal of treatment, better results will be achieved. However, because complete regression of complex atherosclerotic lesions is not currently achievable, the inescapable conclusion is that the future effort should be directed toward the primary prevention of CAD starting in adolescence, especially in individuals at high risk.

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References


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