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LOWERING PLASMA CHOLESTEROL BY RAISING LDL RECEPTORS

More than 93 per cent of the body's cholesterol is located in cells, where it performs vital structural and metabolic functions; only about 7 per cent circulates in plasma, where it predisposes to atherosclerosis. All the cholesterol in plasma is packaged within lipoprotein particles; two thirds is in low-density lipoprotein (LDL). Epidemiologic data and animal experiments indicate that plasma LDL is a major cause of atherosclerosis, particularly in the one of every 500 members of the population who has familial hypercholesterolemia. Because of their elevated LDL levels, male heterozygotes with this dominant disease have an 85 per cent chance of sustaining a myocardial infarction before the age of 60. Female heterozygotes also have a markedly increased risk.[1]

The reason for the elevated LDL levels in familial hypercholesterolemia became apparent several years ago, when subjects with the disorder were found to have a defect in the gene for the LDL receptor.[2] Normal cells produce this surface receptor when they require cholesterol for synthesis of new membranes, bile acids, or steroid hormones. Plasma LDL binds to the receptor and is taken into the cells and degraded, yielding its cholesterol for use in cellular metabolism. Heterozygotes with familial hypercholesterolemia have only one functional gene for the LDL receptor, and their cells therefore synthesize only half the normal number. In the body, heterozygotes compensate for having half the normal number of receptors by doubling their plasma LDL level. In the steady state, they degrade a normal amount of LDL through the receptor, but at the price of a twofold elevation in LDL levels — a situation that eventually leads to atherosclerosis.[1]

The goal of therapy in familial hypercholesterolemia is to reduce the concentration of LDL in plasma without disrupting cholesterol delivery to cells. The ideal approach is to stimulate the cells to produce more LDL receptors. When the number of receptors increases, the rate of LDL degradation will also initially increase. If the rate of LDL production does not change, the LDL level must decline. As the LDL level declines, the rate of LDL degradation falls, since the rate of receptor binding is proportional to the LDL concentration. Eventually a new steady state is attained, in which the absolute rates of LDL degradation and cholesterol delivery to cells are the same as they were initially (and are equal to the rate of LDL production), but in which the plasma LDL concentration has fallen approximately in proportion to the increase in LDL receptors. This new steady state is manifested as an increase in the fractional catabolic rate for LDL, which is the absolute rate of LDL degradation (in milligrams per day) divided by the

Since in the steady state the catabolic rate must equal the production rate, the LDL production rate ultimately determines the amount of cholesterol delivered to tissues by LDL. The number of LDL receptors does not affect the amount of cholesterol delivered; rather, it determines the plasma level of LDL at which this delivery will occur, and it determines which tissues will take up LDL. To lower plasma LDL, it may not be necessary to decrease the amount of cholesterol transported; it may be sufficient to increase the efficiency of transport by raising the amount of LDL receptors. For example, an animal such as the dog, which has a plasma LDL-cholesterol level of 20 mg per deciliter, produces as much LDL per kilogram of body weight per day as does a man with an LDL-cholesterol level of 80 mg per deciliter.[1,3] The same amount of LDL cholesterol is delivered to tissues. The difference in plasma levels is due to the high fractional catabolic rate[3] in the dog (1.6 pools per day) as compared with the rate[1] in normal men (0.4 pools per day). This rapid turnover is presumably due to the presence of a larger number of LDL receptors in the dog.

How can we increase the number of LDL receptors in human beings? The production of these receptors is known to be regulated by hormonal and metabolic factors.[4] Many agents that affect plasma cholesterol levels act by altering the number of LDL receptors, thereby changing the fractional catabolic rate for LDL. For example, thyroid hormone increases LDL receptors, which explains the classic findings of low plasma cholesterol levels in hyperthyroidism and high levels in hypothyroidism.[5] In rabbits and dogs, a high-cholesterol diet decreases LDL receptors in the liver — a regulatory response that contributes to diet-induced hypercholesterolemia.[4]

Pharmacologically, the production of LDL receptors can be stimulated by resins such as cholestyramine or colestipol, which bind bile acids in the intestine and prevent their normal reabsorption. The liver responds by converting more cholesterol to bile acids, thus tending to lower the hepatic content of cholesterol. To obtain additional cholesterol, the liver mounts a dual response: it increases the synthesis of cholesterol by increasing the activity of a rate-controlling enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase)[6]; and it produces a larger number of LDL receptors, increasing the fractional catabolic rate for LDL and causing plasma LDL levels to fall.[3,7,8] Heterozygotes with familial hypercholesterolemia can respond to cholestyramine because the single normal receptor gene is stimulated to produce additional LDL receptors, thereby increasing the fractional catabolic rate for LDL.[8] However, the magnitude of this response is disappointingly small because the accelerated hepatic production of cholesterol partly offsets the need for new receptors, causing the liver to produce a submaximal number.

In this context the recent discovery of compactin (also called ML-236B) by Akira Endo, at the Sankyo Drug Company in Tokyo, assumes great importance.[9] Isolated from a penicillin mold, compactin seems to have been designed by nature to be an ideal competitive inhibitor of HMG CoA reductase. The enzyme has a 10,000-fold higher affinity for compactin than it has for the structurally similar substrate HMG CoA. At micromolar concentrations, compactin abolishes cholesterol synthesis in cultured human and animal cells.[10] Recently, a related but even more potent analogue, called monacolin K or mevinolin, has been isolated independently by Endo and by workers at the Merck Sharp and Dohme Research Laboratories in the United States.[11]

Compactin and mevinolin reduce the plasma level of LDL in many animal species[3,9,11] as well as in human beings.[12] High-density lipoprotein (HDL) is much less affected. The mechanism of LDL lowering has been studied so far only in dogs.[3] In this species mevinolin lowers LDL by a dual mechanism: it decreases the rate of LDL production by 50 per cent; and it stimulates the production of LDL receptors in the liver, thereby increasing the fractional catabolic rate for LDL.[3] When given together with colestipol to dogs, mevinolin blocks the compensatory increase in hepatic cholesterol synthesis. As a result, hepatic LDL receptors increase threefold, and there is a remarkable 75 per cent reduction in plasma LDL levels.[3]

In this issue of the *Journal,* Mabuchi et al. report a detailed study of compactin's effects on lipoprotein levels in human beings.[13] They used extremely low doses of compactin, less than one tenth the amount used in the dog studies. Yet they observed a dramatic 29 per cent reduction in plasma LDL levels in subjects with heterozygous familial hypercholesterolemia. Plasma HDL levels did not change.

The important lesson from this study and the previous experience with compactin, mevinolin, and bile acid-binding resins is that normal regulatory mechanisms can be exploited to lower plasma LDL. The liver responds to cholesterol deprivation by increasing LDL receptors. Therefore, plasma LDL levels fall, but cholesterol delivery continues and crucial body stores of cholesterol are not depleted. In view of previous experience with animals and human beings, it seems likely that the fall in plasma LDL will delay the development of atherosclerosis. The availability of compactin should allow direct tests of this hypothesis.

In addition to helping patients with familial hypercholesterolemia, compactin offers hope to the large number of patients whose plasma LDL levels are in the upper range for the population and who are predisposed to atherosclerosis yet do not have familial hypercholesterolemia. The cause of such "multifactorial" hypercholesterolemia is unknown; it may be related indirectly to a high intake of fat and cholester-

ol. Even though such persons eat a high-cholesterol diet, their bodies still synthesize three times more cholesterol than is absorbed from the intestine.[14] Inhibition of cholesterol synthesis with compactin, with or without a bile acid-binding resin, should stimulate their production of LDL receptors and reduce their LDL levels despite continued consumption of a diet rich in cholesterol.

Many hurdles must be overcome before compactin or mevinolin can be accepted as a "penicillin" for hypercholesterolemia. No long-term studies of toxicity have been reported in animals or patients. It is possible that these compounds will produce unexpected side effects and that new analogues will have to be developed. Yet the studies with the parent compounds compactin and mevinolin have established a general principle: interference with cholesterol synthesis can trigger an increase in LDL receptors, thereby reducing LDL levels in plasma without depleting vital body stores of cholesterol. This is indeed encouraging news.

University of Texas
Health Science Center
at Dallas
Dallas, TX 75235

MICHAEL S. BROWN, M.D.
JOSEPH L. GOLDSTEIN, M.D.

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TYPE 2 HERPES SIMPLEX VIRUS AND VULVAR CARCINOMA IN SITU

SQUAMOUS-cell carcinoma in situ (CIS) of the vulva is now being reported at an increasing frequency. Its association with cervical CIS and invasive cancer has been recognized, and a "field effect" has been proposed to explain cases of CIS of the vulva, vagina, and cervix as responses to a common stimulus.[1,2] Furthermore, genital CIS lesions are often multicentric. These disorders are especially alarming to patients, since CIS of the cervix can progress to invasive cancer. One topical and especially noteworthy candidate for the role of a common carcinogenic stimulus is herpes simplex virus Type 2 (HSV2), which has been associated with premalignant and malignant squamous epithelium of the cervix but has yet to be established as an etiologic agent for this disease.[3]

In this issue of the *Journal*, Kaufman and his colleagues provide data to establish an association between HSV2 and CIS of the vulva in nine of 10 patients whose lesions were studied for the presence of HSV2 nonstructural protein antigens.[4] The study is timely, since increased numbers of patients with vulvar CIS have been described and the rate of HSV2 infection has risen dramatically in the United States. Data from the Connecticut Tumor Registry reveal that the adjusted incidence rate (per 100,000 female population) for vulvar CIS rose from 0.02 to 0.81 between the years 1945-1949 and 1975-1979. The estimated incidence of HSV2 (i.e., genital herpes) infection has also increased, although proving the validity of this estimate is even more difficult than establishing the rates of genital CIS.[5] Regarding the latter, one must question whether the increase is a real one or a reflection of more vigorous surveillance of women. Recent studies suggest that as many as 40 per cent of patients with vulvar CIS are 40 years old or younger, and that approximately half are asymptomatic at the time of diagnosis — a sharp contrast to earlier times, when the diagnosis of vulvar cancer was often delayed.[6,7] Indeed, vulvar CIS may qualify as a newly rediscovered entity, since it has now been separated from a confused group of infectious, atrophic, and premalignant lesions.

It is important to remember that the malignant potential of vulvar CIS, in contrast to that of cervical CIS, has yet to be established. Although many patients with invasive cervical cancer may have CIS that can be demonstrated histologically in epithelium adjacent to the cancer, less than 20 per cent of the cases of true invasive vulvar cancer involve demonstrable CIS in adjacent histologic sections.[8] In one large series, progression of CIS to invasive vulvar cancer occurred in only 5 per cent of patients; generally, those at high risk for progression to invasive cancer were either elderly or immunosuppressed.[6,7] Furthermore, spontaneous resolution of vulvar CIS has been observed[1,7]; it was noted in one patient in the series of