Modification of low-density lipoproteins by flavonoids

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Much of the cholesterol that accumulates in atherosclerotic lesions is contained within macrophages and may be derived in humans from low-density lipoproteins (LDL) that are oxidized locally in the lesions [1]. Oxidized LDL becomes recognized by scavenger receptors on macrophages and is internalized rapidly.

We have shown previously that flavonoids, a large group of antioxidants ubiquitous in plants, are potent inhibitors of LDL oxidation, the concentration producing 50% inhibition being 1–20 μM [2]. We report here that two particular flavonoids (myricetin and gossypetin) at a higher concentration can modify LDL by themselves so that macrophages will take it up much faster.

LDL was isolated from normal human plasma and radioiodinated [3]. It was incubated for 24 h usually at 100 μg of LDL protein/ml with flavonoids in Ham’s F-10 medium, Dulbecco’s modified Eagle’s medium (DMEM) or Dulbecco’s phosphate-buffered saline containing 50 μg of gentamicin/ml. The LDL was then diluted to 10 μg of protein/ml and incubated for 24 h with mouse resident peritoneal macrophages in culture in DMEM containing 10% (v/v) fetal calf serum and 50 μg of gentamicin/ml. The uptake of the LDL was assessed by measuring its non-iodide trichloroacetic acid-soluble degradation products in the medium [2]. Myricetin was dissolved in ethanol, which had a final concentration of 1% (v/v).

Figure 1(a) shows that 125I-labelled LDL incubated with 100 μM-myricetin was degraded much faster by macrophages and that the modification was complete in 6 h. Myricetin at 10 μM did not modify LDL in this way. Gossypetin at 100 μM (but not 10 μM) modified LDL, but none of the other flavonoids tested did so.

Flavonoids can act as pro-oxidants as well as antioxidants [4, 5], but 100 μM-myricetin did not cause oxidation of the LDL; in fact, it prevented the small increase in the hydroperoxides in LDL that occurred by autooxidation (Fig. 1b). The levels in LDL of the endogenous antioxidant α-tocopherol are decreased when LDL is subjected to oxidation [5], but they did not fall when LDL was incubated with 100 μM-myricetin.

The degradation by macrophages of myricetin-modified LDL plateaued at 10 μg of LDL protein/ml, suggesting the existence of a high-affinity binding site for it on the cells. The degradation of myricetin-modified 125I-labelled LDL (5 μg of protein/ml) was not competed for by non-labelled native LDL (up to 100 μg of protein/ml) and only by about 25% by non-labelled acetylated LDL (up to 100 μg of protein/ml). It therefore appears that macrophages take up myricetin-modified LDL by a process that is largely independent of their scavenger receptors (which take up acetylated or oxidized LDL).

Abbreviations used: LDL, low-density lipoproteins; DMEM, Dulbecco’s modified Eagle’s medium.

Fig. 1. Time course of the modification of LDL by myricetin
LDL (100 μg of protein/ml) was incubated for up to 24 h with Dulbecco’s phosphate-buffered saline in the presence (●) or absence (○) of 100 μM-myricetin, diluted to 10 μg of protein/ml and incubated for 24 h with macrophages and its degradation by them measured (a). The levels of hydroperoxides in the LDL were measured by an automated triiodide assay [5](b).

LDL was modified much more by 100 μM-myricetin at 100 μg of LDL protein/ml than at 50 μg of protein/ml. Sometimes (but not always) the LDL was visibly aggregated, suggesting that aggregation may be involved in the modification process.

Flavonoids are components of the normal human diet and about 1 g are ingested each day. Little is known, however, about their pharmacokinetics and the concentrations they reach in plasma. It appears doubtful, however, if they would reach sufficient concentrations to modify LDL themselves and so, if they exert effects in vivo, their inhibitory effects in preventing LDL modification by oxidation may predomi-
The effect of 3-hydroxy 3-methylglutaryl-CoA reductase inhibitor on the subfractions of high-density lipoprotein of the rabbit

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To study the development of atherosclerosis and its relation to lipoproteins and cholesterol, a variety of animal models have been used. Rabbits have been fed fats and animal proteins to induce atherosclerosis which resembles the disease process as it occurs in man [1]. The response is obtained in short periods of time and lipid-rich lesions are easily identified in aorta and coronary arteries of rabbits. The rabbit is also very sensitive to 3-hydroxy 3-methylglutaryl-CoA reductase (HMG-CoA reductase) inhibitors [2], the drug used in the present study.

HMG-CoA reductase is an enzyme required for the synthesis of cholesterol from acetyl-CoA [3]. Approximately two-thirds of plasma cholesterol derives from this synthesis [4]. To reduce the levels of cholesterol in hyperlipidaemic patients, and therefore the risk of atherosclerosis, many inhibitors of this enzyme are being tested for clinical use. These inhibitors are known to decrease the concentration of total plasma cholesterol, low-density lipoproteins (LDL) and, in some cases, very-low-density lipoproteins (VLDL) [5]. They are also known to increase total high-density lipoproteins (HDL) in some patients [6], but their effect on the subfractions of HDL remains unknown. Specific subfractions of HDL are involved in reverse cholesterol transport and have a protective role against atherosclerosis.

In the present study, we have therefore analysed the subfractions of HDL of the normolipidaemic rabbit submitted to treatment with Simvastatin, a potent HMG-CoA reductase inhibitor.

Six female New Zealand White rabbits were fed a standard laboratory rabbit chow ad libitum. The normal lipoprotein profile and plasma cholesterol and triacylglycerol concentrations were determined over a 6 week period before administration of Simvastatin (10 mg/day per kg), dissolved in ether and sprinkled over the food, for a further 8 week period.

Blood samples (20 ml) were drawn from marginal ear veins and collected in EDTA vials after 2 and 5 weeks from the start of the study and after 1, 3, 5 and 8 weeks of introducing Simvastatin to the diet.

VLDL, LDL and HDL were isolated by ultracentrifugation at densities of 1.02 g/ml, 1.055 g/ml and 1.25 g/ml, respectively. An aliquot of each HDL sample obtained was submitted to gradient polyacrylamide-gel electrophoresis on Pharmacia PAA 4/30 gels and the resulting subfractions scanned and quantified using the BioRad 620 video densitometer.

Abbreviations used: HMG-CoA reductase, 3-hydroxy 3-methylglutaryl-CoA reductase; LDL, low-density lipoproteins; VLDL, very-low-density lipoproteins; HDL, high-density lipoproteins.

A further portion of each HDL sample was exhaustively dialysed for removal of NaBr and applied to Heparin-Sepharose affinity chromatography column, for the separation of apo-E-poor and apo-E-rich HDL [7, 8].

All the animals survived the study, but one rabbit was withdrawn at the eighth week of drug treatment owing to anorexia. [The following results are expressed as mean value ± S.D. (n = 6, except for week 8, when n = 5)]. The mean concentration of total plasma cholesterol decreased from 78.4 ± 33.5 mg/dl before treatment to 31.2 ± 8.9 mg/dl after the 8 week period when the rabbits were given Simvastatin. The action of the drug was rapid, since a decrease of 36% in the total plasma concentration of cholesterol was observed after the first week of treatment. The mean concentration of total plasma triacylglycerol varied from 84.8 ± 30.7 mg/dl before treatment to 82.5 ± 7.2 mg/dl after Simvastatin.

The mean concentration of LDL-cholesterol decreased from 45.5% of total lipoprotein-cholesterol to 14.7% with drug treatment.

During the first 3 weeks of Simvastatin administration, the concentration of total plasma HDL-cholesterol increased from 40.4% to 70.5% of total lipoprotein-cholesterol, while the concentration of VLDL decreased from 14.1% to 8.7% of total lipoprotein-cholesterol. During the subsequent 5 weeks, the concentration of HDL-cholesterol decreased to a level that was 22.8% of the total lipoprotein-cholesterol and that of VLDL increased to 62.5% of the total.

There was a significant difference in the proportion of apo-E-rich and apo-E-poor HDL with Simvastatin treatment. The apo-E-rich HDL level decreased from 58.4% ± 11.4 of total HDL, before drug was started, to 46.1% ± 8.66 (P < 0.01) after treatment. The profile of HDL subfractions observed after resolution on gradient-gel electrophoresis showed no significant change with drug treatment.

The inverse relationship observed between the concentrations of VLDL-cholesterol and HDL-cholesterol together with the changes in the concentration of VLDL-cholesterol on treatment is suggestive of changes in the activity of lipoprotein lipase, which hydrolyses the triacylglycerol moiety of VLDL with the production of HDL of large particle size [9] containing apo E [10]. However, the observed lack of any change in the distribution of HDL subfractions of different size, as determined by gradient-gel electrophoresis, would indicate that alternative or additional mechanisms are operative. Present studies in this laboratory have demonstrated that reduced levels of HDL₃ₙ and apo-E-rich HDL are present in the plasma of subjects of high coronary risk [8] and in survivors of myocardial infarction [11]. The observation made in the present study that the levels of the corresponding HDL subfractions are maintained during treatment with Simvastatin might suggest that the protective role of HDL against coronary heart disease is preserved during administration of this drug.

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