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Incorporation of lycopene into chylomicron remnant-like particles inhibits their uptake by HepG2 cells

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Abstract

The influence of the incorporation of the antioxidant tomato pigment, lycopene, into chylomicron remnant-like particles (CRLPs) on their uptake by the liver cells was investigated. CRLPs or CRLPs containing lycopene (lycCRLPs) radiolabelled with [^{3}H]triacylglycerol were incubated with cells of the human liver hepatoma cell line, HepG2, and the radioactivity taken up by the cells was determined. LycCRLPs were taken up significantly more slowly than CRLPs over a concentration range of 5–60 μg cholesterol/ml and a time course of 2–6 h. Pre-incubation of the hepatocytes with an excess of low density lipoprotein (LDL) inhibited the uptake of CRLPs by about 50%, but had no effect on the uptake of lycCRLPs, and under these conditions the CRLPs and lycCRLPs were taken up at similar rates. In HepG2 cells pre-treated with suramin, which inhibits uptake via the LDL receptor-related protein (LRP), the uptake of CRLPs was also inhibited (−37%) to a greater extent than that of lycCRLPs (−24%), so that the values for the two types of particle were no longer significantly different. Heparinase increased the uptake of lycCRLPs (about 2 fold), but not CRLPs, bringing it to a level equivalent to that seen with the control particles. These findings demonstrate that the incorporation of lycopene into CRLPs decreases their uptake by HepG2 cells and suggest that this effect is due to differential interaction with the LDL receptor and the LRP-receptor-mediated pathways, and may also involve binding of the particles to HSPG.
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Keywords: Chylomicron remnants; Lycopene; Dietary fat; LDL receptor; LDL receptor-related protein; HepG2 cells

Introduction

Atherosclerotic cardiovascular disease, the leading cause of morbidity and mortality in developed countries, is multifactorial, and as well as environmental and genetic factors, the composition of the diet is known to modulate lesion development, with both the amount and type of fat and the content of micronutrients playing a significant role (Yusuf et al., 2001). Atherosclerosis begins with the appearance of fatty streaks in the artery, which occur when macrophages which have invaded the vessel wall become engorged with lipid and form foam cells (Libby et al., 1996). The lipid is derived from the plasma lipoproteins, particularly low density lipoprotein (LDL), but oxidative modification of LDL is necessary before foam cell formation is induced (Albertini et al., 2002), and this has led to the idea that dietary antioxidants may be beneficial in preventing coronary heart disease.

A large amount of evidence from epidemiological, animal and in vitro studies suggests that diets rich in fruit and vegetables (for example, the Mediterranean diet) containing relatively high levels of natural antioxidants such as plant carotenoids may be protective against atherosclerosis (Kaliora et al., 2005; Trichopoulou and Vasilopoulou, 2000; Rao, 2002). Despite this, however, large scale trials of dietary supplementation with antioxidants such as β-carotene or vitamin E in healthy human populations have failed to show any benefit (Kaliora et al., 2005; Clarke and Armitage, 2002).

After absorption in the intestine, dietary lipids, including lipophilic antioxidants, enter the blood via the lymph in chylomicrons. These large triacylglycerol (TG)-rich lipoproteins are then rapidly lipolysed to remove some of their TG. The resulting smaller chylomicron remnants retain all the cholesterol and

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minor lipid components such as antioxidants, and deliver them to the liver for processing (Redgrave, 1983). There is now compelling evidence to indicate that these chylomicron remnant particles are strongly atherogenic; they can be taken up into the artery wall as efficiently as LDL (Proctor et al., 2002; Grieve et al., 1998); chylomicron remnant-like particles have been isolated from human atherosclerotic plaque (Pal et al., 2003; Rapp et al., 1994); and their delayed clearance from the circulation is correlated with atherosclerotic lesion development (Benlian et al., 1996; Groot et al., 1991). Furthermore, studies in our laboratory and others have demonstrated that chylomicron remnants induce foam cell formation in macrophages (Batt et al., 2004; Botham et al., 2005). Our previous work has also shown that, in striking contrast to LDL, chylomicron remnants do not require prior oxidation to bring about this effect, and surprisingly, that incorporation of antioxidants such as lycopene (a carotenoid found in tomatoes) or the lipophilic drug probucol into the particles increases their rate of uptake by macrophages, and thus enhances, rather than inhibits, foam cell formation (Moore et al., 2003, 2004). This unexpected effect of chylomicron remnants could clearly promote lesion development in the vasculature, but more rapid clearance of the particles by the liver may be advantageous, as the time available for interaction with the artery wall would be reduced. The aim of the present study was to test the hypothesis that incorporation of the dietary antioxidant lycopene into chylomicron remnants increases their rate of uptake by liver cells.

The hepatic uptake of chylomicron remnants is mediated by the LDL receptor (LDLr) and the LDLr-related protein (LRP) (Rohlmann et al., 1998; Yu and Cooper, 2001). Initial sequestration by cell surface heparan sulphate proteoglycans (HSPG) is believed to be important, particularly in internalisation via the LRP (Yu and Cooper, 2001; Zeng et al., 1998) and lipoprotein lipase (LPL) is also thought to act as a ligand to facilitate the process (Zeng et al., 1998). In this study, we have examined the effects of incorporation of lycopene into chylomicron remnant-like particles (CRLPs) on their uptake by the human hepatoma cell line HepG2. Contrary to our hypothesis, the results suggested that the CRLPs containing lycopene (lycCRLPs) were taken up by HepG2 cells at a slower rate than CRLPs without lycopene, and we therefore carried out further studies using selective inhibitors of hepatic remnant uptake and other factors known to influence the process to investigate the mechanisms involved.

Materials and methods

Materials

Glycerol-tri[9,10(n)-^{3}H]oleate (28.0 Ci/mmol) and [4-^{14}C] cholesteryl oleate were purchased from Amersham Pharmacia Biotech Inc. (Milan, Italy). HepG2 cells were obtained from ATCC, Minimum Essential Medium/Earle's salt (MEM/EBSS) glutamine, non-essential L-amino acids, sodium pyruvate, fetal bovine serum (FBS), trypsin, penicillin and streptomycin were obtained from Euroclone Ltd. Lycopene, suramin, heparinase, fatty acid-free bovine serum albumin (BSA), and various classes of lipids and solvents were purchased from Sigma Chemical Company (St. Louis, MO, USA). LDL from pooled normolipidemic human plasma was prepared by ultracentrifugation as previously described (Napolitano and Bravo, 2003).

Preparation of CRLPs

CRLPs radiolabelled with [^{3}H]triolein were prepared by sonication of a lipid mixture followed by density gradient ultracentrifugation as described by Diard et al. (1994). Briefly, a lipid mixture (70% trilinolein or triolein, 2% cholesterol, 5% cholesteryl ester, 25% phospholipids) containing glycerol-tri [^{3}H]oleate (3.7 MBq) in Tricine buffer (20 mM, pH 7.4) containing 0.9% NaCl was sonicated at a power setting of 22–24 μm for 20 min at 56 °C. The resulting emulsion was adjusted to a density of 1.21 g/ml with KBr, layered under a step-wise density gradient and centrifuged at 17,000 *g* for 20 min at 20 °C. The upper layer of grossly emulsified lipids was then removed and replaced with an equal volume of 0.9% NaCl (d=1.006 g/ml) and centrifuged at 70,000 *g* for 1 h (20 °C). The top layer was then harvested. Human apoE was bound to the lipid particles obtained by incubation (37 °C, 18 h, with shaking) with the dialysed (0.9% NaCl, 18 h, 4 °C) d>1.006 g/ml fraction of fasting human plasma in a ratio of 1:2.5 (v/v). CRLPs were then isolated by ultracentrifugation (45,000 *g*, 18 h, 12 °C), harvested from the top layer and stored at 4 °C under argon until required. Analysis by SDS PAGE showed that the particles contained apoE and no other lipoproteins. CRLPs containing lycopene (lycCRLPs) were obtained by addition of the compound (1 mg) to the lipid mixture prior to sonication. Taking into account the amount of lycopene added to the lipid mixture for sonication and the percentage recovery of the starting lipids in the CRLPs, the final concentration of lycopene used in our experiments did not exceed 10 μM at a concentration of CRLPs of 30 μg cholesterol/ml.

Maintenance and culture of HepG2 cells and experimental protocols

HepG2 cells were cultured in tissue culture flasks at an initial density of 3×10^{4} cells/cm^{2} in MEM/EBSS medium containing 10% (v/v) FBS, 2% (w/v) glutamine, 100 units/ml penicillin and 100 mg/ml streptomycin. The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air and the medium was changed every 2 days. At 80% confluency, the cells were trypsinized with 0.1% trypsin and 0.02% EDTA in PBS and then subcultured in 24-well plates for the experiments. The cells were incubated with CRLPs or lycCRLPs at a concentration of 30 μg cholesterol/ml for 4 h (except where indicated otherwise). The medium was then removed, the cells were washed (1×3 ml PBS) and the lipids were extracted as described below. In some experiments, the cells were pre-incubated with LDL (100 or 400 μg cholesterol/ml), suramin (500 μg/ml), trypsin (0.05% w/v) or heparinase (2 U/ml) for 1 h prior to addition of the CRLPs. The viability of the cells as assessed by Trypan blue exclusion

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