Oligomeric Proanthocyanidins effectively inhibit LDL oxidation: A Mechanistic study using a lipophilic, Oxidation-sensitive, Fluorescent Probe

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Introduction

Atherosclerosis is a chronic inflammatory disease, and constitutes the leading cause of morbidity and mortality in the Western world. Reactive oxygen species (ROS) have been widely implicated as a causative factor in atherosclerosis. ROS promote atherosclerosis by inducing vascular endothelial cell dysfunction, oxidation of LDL, and inflammation. Targeting ROS with dietary antioxidants is therefore an attractive approach for preventing atherosclerosis, and this approach is supported by epidemiological evidence correlating dietary antioxidant intake with lower risk of cardiovascular disease. Oligomeric proanthocyanidins (OPCs) are complex, readily bio-available phytonutrients, composed of oligomers of 2 to 5 flavan-3-ol (catechin) units, whose presence in the diet is compromised by the fact that OPCs are mostly found in discarded food parts such as skins and seeds. The specific OPCs-Compound used in the present study was shown in previous human intervention studies to have significant beneficial effects on vascular function. This OPCs-Compound was also found to exhibit strong antioxidant activity, and to protect vascular endothelial cells from oxidative damage in vitro.
In the current study, we investigated if the specific OPCs-Compound could inhibit LDL oxidation and thereby find potential application in atherosclerosis prevention.

Material and Methods

Human LDL was isolated [1] and labelled with1 µM C11-Bodipy$^{581/591}$, a fluorescent lipophylic probe whose fluorescence shifts from red to green upon oxidation (Figure 1). Subsequently LDL (200 µg/ml in HBSS) was subjected to Cu^{2+} or MeO-AMVN to induce lipid peroxidation, which was monitored on-line. Additional experiments were done using small unilamellar vesicles consisting of dilinoleoylphosphatidyl choline containing 0.05% C11-Bodipy$^{581/591}$.

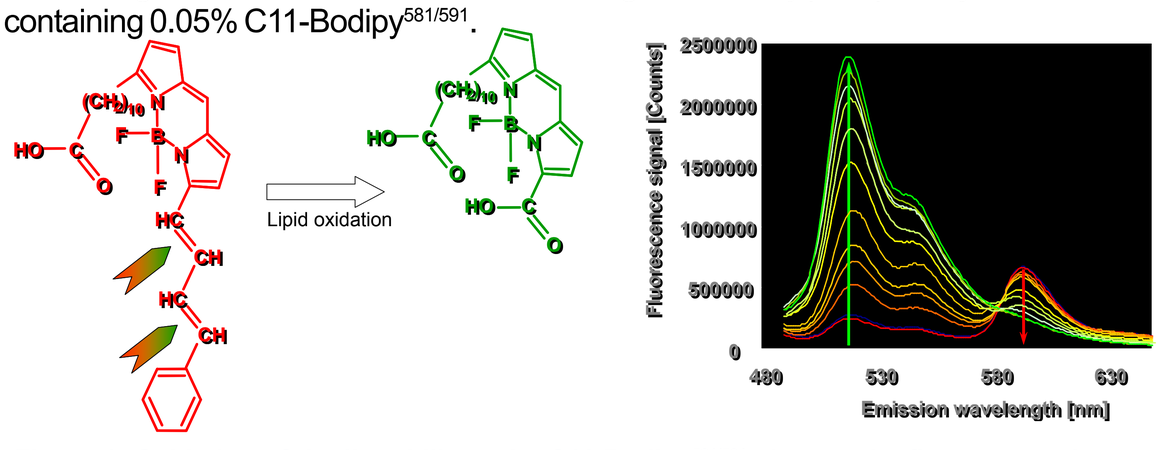

Figure 1: Oxidation of the lipophilic probe C11-Bodipy$^{581/591}$ changes its fluorescent characteristics, which shift from red to green. The left panel shows the molecular mechanism [2] and the right panel shows the shift in the emision upon oxidation. The fluorescent signal can be monitored on-line and is suitable for multiwell technology and imaging [3,4].

Results

Analysis of the OPCs-Compound by HPLC (Figure 2):

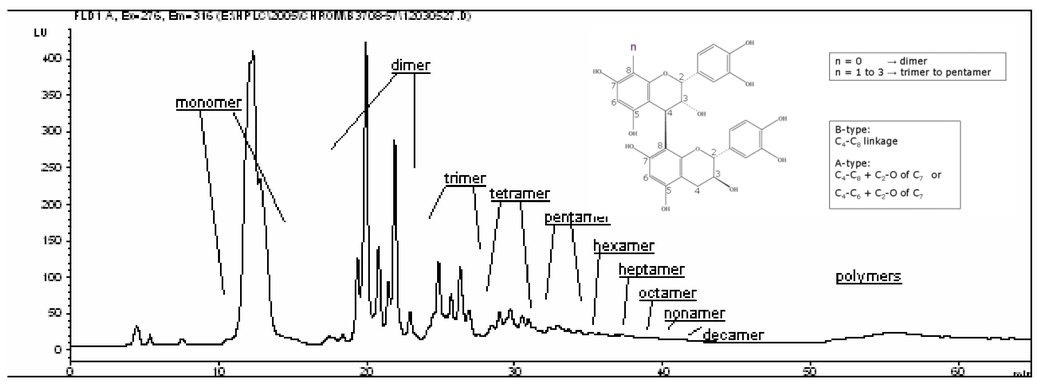

Figure 2: The OPCs-Compound was analyzed by HPLC. As the chromatogram in Fig.2 indicates, the product comprises of catechins and oligomers of 2-5 flavan-3-ol units. Catechins and dimeric forms of flavan-3-ols accounted for over 40% of the total product. The remaining flavan-3-ols were trimers, tetramers and pentamers. The extraction process ensured that clusters of 6 or more flavan-3-ol units formed less than 1% of the product..

Detection of hLDL oxidation using BODIPY (Figure 3):

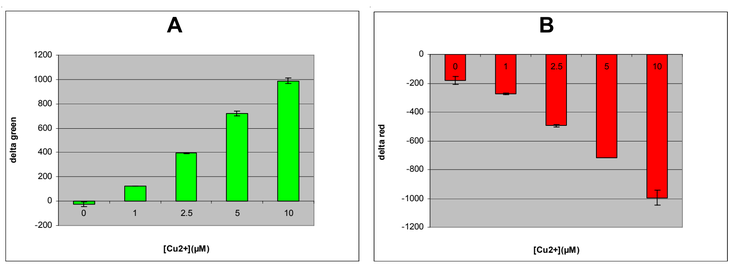

Figure 3: Exposure of BODIPY–labelled hLDL to Cu^{2+} (0-10 µM) resulted in concentration-dependent increase in green fluorescence (A) and decrease in red fluorescence (B), indicating sensitivity and specificity of the C11-Bodipy $^{581/591}$probe to hLDL oxidation

OPCs Protect hLDL from Cu^{2+}-induced oxidation (Figure 4):

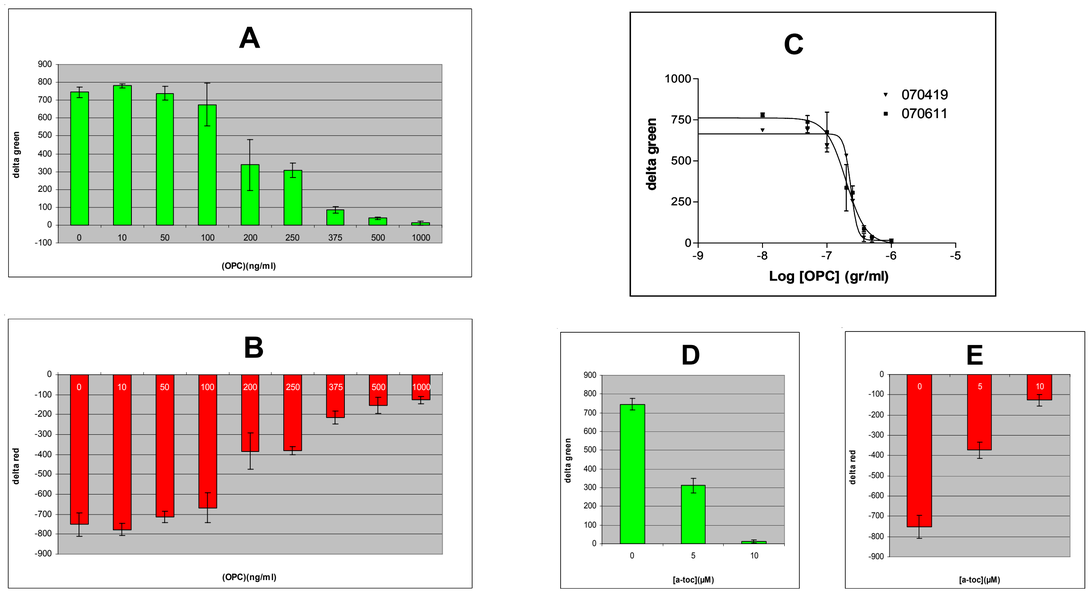

Figure 4: OPCs inhibited Cu^{2+}-induced hLDL oxidation: (A/B) Pre-incubation of labelled hLDL with OPCs (0-1000 ng/ml) inhibited Cu^{2+}-induced oxidation, seen by the reduction of the delta green signal and delta red signal. (C) EC_{50} of OPCs is 216 ng/ml whereas (D) and (E) higher alpha-tocopherol concentration (5 µM) was required to inhibit Cu^{2+} -induced hLDL oxidation

OPCs Protect hLDL from Lipophilic MeO-AMVN induced oxidation (Figure 5):

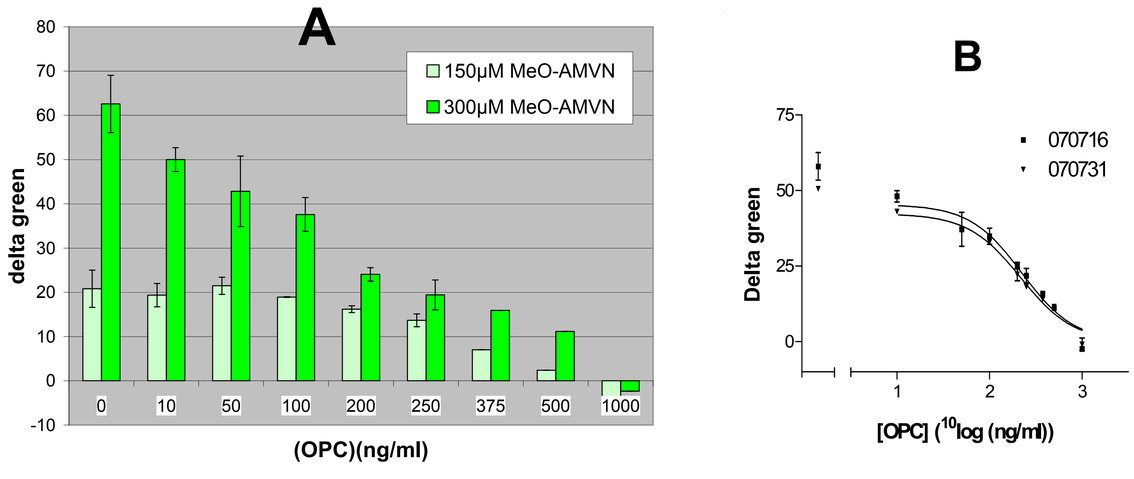

Figure 5: OPCs inhibited hLDL oxidation induced by lipophilic radical-generator MeO-AMVN: (A) MeO-AMVN caused oxidation of hLDL, particularly at a concentration of 300µM. Pre-incubation of hLDL with OPCs (0-1000 ng/ml) resulted in inhibition of MeO-AMVN-induced oxidation as seen by a decrease in the green signal. (B) The EC_{50} for inhibition of MeO-AMVN-induced hLDL oxidation was 221ng/ml of OPCs.

Potential Mechanism for Inhibition of hLDL Oxidation by OPCs (Figure 6):

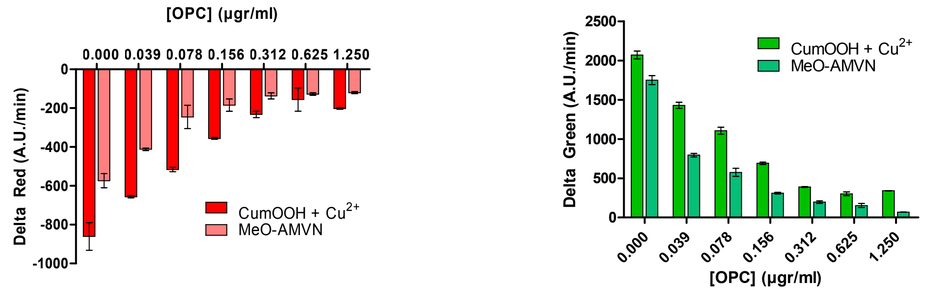

Figure 6: Inhibition of lipid peroxidation in vesicles by OPCs: Synthetic lipid vesicles containing C11-Bodipy$^{581/591}$ were exposed to the lipophilic radical-generator Meo-AMVN, which is incorporated within the lipidbilayer, or to cumene hydroperoxide + Cu^{2+}. In both conditions OPCs significantly inhibited the peroxidation process. This indicates that OPCs inhibit the lipid peroxidation process not only by scavenging radicals in the waterphase, but also by acting within or at the level of the lipid bilayer.

Summary and conclusion

- **A specific OPCs-compound used in this study (at concentrations that reflect biological relevant in vivo levels) significantly attenuated oxidation of hLDL induced by Cu^{2+} and by the lipophilic-radical generator MeO-AMVN.**
- **The OPCs-compound also inhibited oxidation of synthetic lipid vesicles induced by cumene hydroperoxide + Cu^{2+} Meo-AMVN, indicating that OPCs likely inhibited lipid peroxidation by (i) scavenging radicals in the water phase and (ii) interupting of the lipid peroxidation reaction within lipid bilayers.**
- **In conclusion, the results reveal a plausible mechanism by which OPCs inhibit LDL oxidation, and provide a basis for further investigating the potential protective effects of OPCs in atherosclerosis.**

[1] Redgrave et al, Anal. Biochem. 65: 42 (1975). [2] Drummen et al, Free Rad. Biol. Med. 36: 1635 (2004). [3] Drummen et al, Free Rad Biol Med 33: 473(2002). [4] Bapat et al, J Am Soc Nephrol 12: 2990 (2002).