

## Introduction

Reactive oxygen species (ROS) are involved in the pathology of cardiovascular conditions such as inflammation, atherosclerosis etc. Endothelial cells, highly specialized cells, line the vasculature and thereby form a selective barrier between the circulating blood and the underlying parts of the vasculature. ROS damage to endothelial cells results in endothelial dysfunction, which plays an important role in the onset and progression of vascular disease. Therefore, controlling ROS is an attractive approach towards preventing cardiovascular disease. Epidemiologic studies have demonstrated an association between increased intakes of antioxidants and reduced mortality from coronary heart disease. Of particular interest are the antioxidants termed as oligomeric proanthocyanidins (OPCs). They are composed of condensed clusters of 2 to 5 single flavan-3-ol (catechin) units (see insert figure 2). Unlike many synthetically manufactured nutrients, OPCs are derived from various plant sources in widely varying qualities. Clinical studies have shown beneficial effects on vascular function of the specific OPCs-compound used in the present study. We investigated the ability of these oligomeric proanthocyanidins (OPCs) to protect vascular endothelial (VE) cells from lipid oxidation.

## Material and Methods

Fetal bovine heart endothelial cells were grown in Dulbecco's Modified Eagle's Medium supplemented with 7.5 % FBS at 37°C in a 5% CO<sub>2</sub> humidified atmosphere. The OPCs-rich product, isolated from *Vitis vinifera* seeds was provided by International Nutrition Company. No effects were induced by the vehicle ethanol. The VE cells were grown for 24 hrs in the presence of a range of OPCs, after which the medium was washed-off. Cells were labeled with C11-Bodipy<sup>581/591</sup>, a fluorescent lipophilic probe whose fluorescence shifts from red to green upon oxidation (Figure 1). Subsequently the cells were subjected to varying doses of cumene hydroperoxide (CumOOH) to induce lipid peroxidation, which was monitored on line. Next to this we studied the effect of OPCs in a lipid vesicle system, consisting of DiIinoeoylPC vesicles containing 0.05% C11-Bodipy<sup>581/591</sup>. The latter experiments were carried out at lower concentrations of OPCs, and using another lipid peroxidation inducing stress (AMVN), in addition to CumOOH/Cu<sup>2+</sup>.

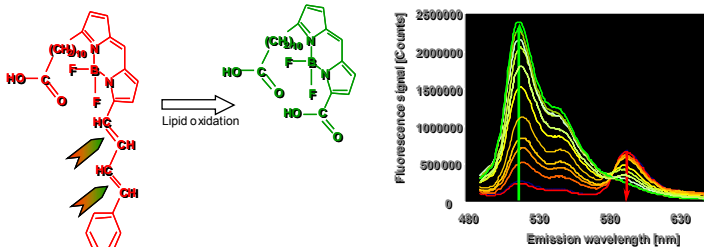


Figure 1: Oxidation of the lipophilic probe C11-Bodipy<sup>581/591</sup> changes its fluorescent characteristics, which shift from red to green. The left panel shows the molecular mechanism [1] and the right panel shows the shift in the emission upon oxidation. The fluorescent signal can be monitored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

## Results

Figure 2 presents the composition of the product used in this study as verified by HPLC. The chromatogram showed that the product comprised catechins and oligomers of 2-5 flavan-3-ol units. Catechins and dimeric forms of flavan-3-ols were most common and accounted for over 50% of the total product. The remaining procyanidins 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.

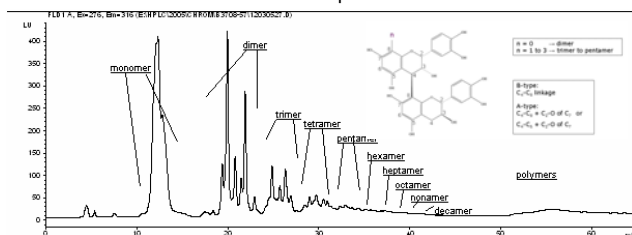


Figure 2: HPLC analysis of the OPCs product used. The chromatogram shows that the product comprised catechins and oligomers of 2-5 flavan-3-ol units. Catechins and dimeric forms of flavan-3-ols were most common and accounted for over 50% of the total product. The remaining procyanidins were trimers, tetramers and pentamers. The extraction process ensured that clusters of 6 or more flavan-3-ol units formed less than 1% of the product.

Exposing the endothelial cells to CumOOH/Cu<sup>2+</sup> resulted in a dose dependant increase in lipid oxidation in the cells (Figure 3).

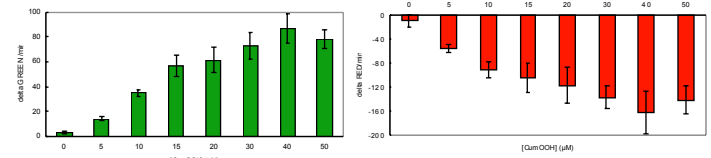


Figure 3: Lipid peroxidation in VE cells during exposure to CumOOH. The left panel shows the rate of increase of green signal and the right panel shows the decrease in the red signal.

Pre-incubation of cells for 24 hours with the OPCs protected the cells dose dependently from CumOOH induced lipid peroxidation (Figure 4).

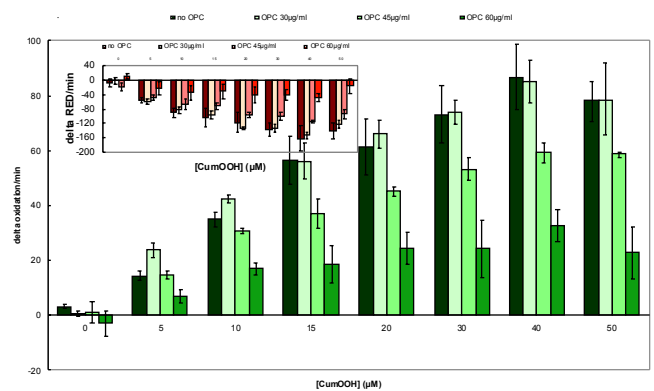


Figure 4: Lipid peroxidation in VE cells during exposure to CumOOH after pretreating the cells with OPCs. The large panel shows the rate of increase of green signal and the insert shows the decrease in the red signal.

To assess whether the effect of OPCs on lipid peroxidation was dependent on the cellular environment/setting or whether it was a direct effect we studied the effect of OPCs in a lipid vesicle system. Two forms of stress (CumOOH/Cu<sup>2+</sup> and AMVN) were used in separate experiments. The results are presented in Figure 5. In both these systems, the presence of OPCs resulted in a dose dependent attenuation of the oxidation was observed

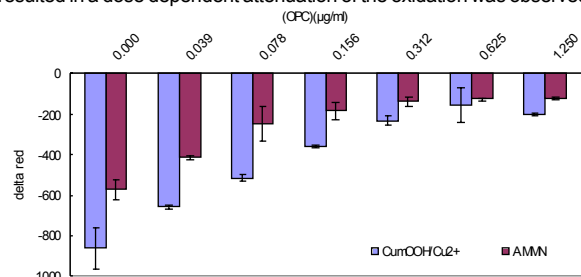


Figure 5: Lipid peroxidation in DiLPC vesicles during oxidative stress induced by CumOOH/Cu<sup>2+</sup> or AMVN in the presence of a range of OPCs. The panel shows the decrease in the red signal.

## Conclusion

In conclusion, the results demonstrate that the OPCs used in the present study, protect against the oxidation of lipids in vascular endothelial cells and in artificial lipid systems.

## Acknowledgements

We would like to thank Dr. D. van der Vlies for the HPLC data and the International Nutrition Company for financial support.

[1] Drummen et al., Free Rad. Biol. Med. 36: 1635-1644 (2004). [2] Drummen et al., Free Rad Biol Med 33: 473-490 (2002). [3] Bapat et al., J Am Soc Nephrol 12: 2990-2996 (2002).