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Atherosclerosis regression study in rabbits upon olive pomace polar lipid extract administration

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Abstract *Background and aims:* Virgin olive oil polar lipid extract (OOPL) and olive pomace polar lipid extract (PPL) have similar antiatherosclerotic effects in cholesterol-fed rabbits. Our aim was to compare the effect of PPL with that of simvastatin on the progression of atherogenesis.
Methods and results: Rabbits were fed an atherogenic diet for 6 weeks in order to develop dyslipidemia and atheromatous lesions. Following documentation of these events in random animals (group A, $n = 6$), the remaining were fed for 3 weeks with: standard chow alone (group B, $n = 6$), chow supplemented with PPL (group C, $n = 6$), and chow supplemented with simvastatin (group D, $n = 6$). Blood was collected at 0, 6 and 9 weeks, to determine plasma lipid levels, plasma PAF-AH activity, platelet aggregation (PAF-EC_{50}), resistance of plasma to oxidation (RPO) and extent of atheromatous lesions in aortas. The atherogenic diet induced dyslipidemia and increased PAF-AH activity. Dyslipidemia and PAF-activity reduced more effectively

Acronyms: OOPL, Polar lipid extract of virgin olive oil; PPL, Polar lipid extract of olive pomace; CVD, Cardiovascular diseases; DPPH-EC_{50}, Equivalent concentration that scavenges $DPPH^{\cdot}$ by 50%; PAF, 1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine; PAF-AH, PAF-acetylhydrolase; PAF-EC_{50}, Equivalent concentration that reduces PAF-induced platelet aggregation by 50%; PRP, Platelet-rich plasma; PPP, Platelet-poor plasma; BSA, Bovine serum albumin; Tris, Hydroxymethyl aminomethane; TCA, Trichloroacetic acid; IC_{50}(PAF), Concentration that inhibits PAF-induced aggregation by 50%; IC_{50}(PAF-R), Concentration that antagonizes PAF-binding to its receptor by 50%; PBS, Phosphate buffer solution; TC, Total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TAG, Triacylglycerol; MUFA, Mono-unsaturated fatty acid; PUFA, n-3 poly-unsaturated fatty acid; PAF-R, PAF receptor; VOO, Virgin olive oil.

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in groups C and D. RPO decreased in group B only. PAF-EC_{50} values decreased in group C only. Atherogenesis progression in group C was prevented to an extent indistinguishable from that in group D. PAF-AH activity was positively correlated, whereas RPO was negatively correlated with the extent of atheromatous lesions.
Conclusion: PPL, as a dietary supplement, is equipotent to simvastatin in preventing the progression of atherogenesis.

Introduction

Cardiovascular diseases (CVD) are the major cause of death in the Western world; therefore inhibiting atherosclerosis development has gained popularity in treating CVD. On the other hand, atherosclerosis regression, a more complicated process than atherosclerosis development, has not received much attention. In the rabbit model of atherosclerosis it is difficult to induce atherosclerosis regression by replacing an atherogenic diet with standard chow [1,2]. However, atherosclerosis regression in rabbits has been reported using synthetic and naturally derived substances [3—7], such as statins. The findings of statin administration studies led to their wide use as drugs to control serum cholesterol levels and to treat CVD [8].

Olive oil administration inhibits atherosclerosis development [9,10], but its effects on atherosclerosis regression are not yet clear [11,12]. Recent reports suggest that olive oil's beneficial role is not entirely linked to its high oleic acid content, but also to its microconstituent content, such as tocopherols, phenolic compounds, phytosterols, triterpenoids and unusual glycolipids exerting an antagonistic effect on PAF (1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) [13—16]. Microconstituents of olive pomace, similar to those of olive oil, may also have beneficial effects on the outcomes of CVD [13,15,16].

We have reported that atheromatous lesion development was inhibited in rabbits by supplementation of an atherogenic diet with either virgin olive oil or OOPL [10]. OOPL and PPL contain glycolipids and a few phenolics that act as PAF-antagonists [17]. Since there are no *in vivo* studies reporting the effect of PPL on atherosclerosis regression the aim of this study was to investigate this effect on the rabbit model of atherosclerosis.

Methods

PPL extract

Isolation of PPL from olive pomace was carried out using the modified counter current distribution method previously reported [17]. PPL characteristics are presented in Fig. 1B.

Animal diets and handling

Twenty-four male New Zealand rabbits (2.7 ± 0.3 kg, 2.5 weeks old) were purchased from a local commercial breeder and were individually housed in an air conditioned room (12 full changes of air per 1 h), 19 ± 1 °C temperature, 55 ± 5% relative moisture and light/darkness ratio 12 h/12 h. Living conditions and animal handling were carried out according to European Regulation 609/86, as certified and approved by the local veterinary authorities and animal ethics committee. Rabbits were acclimatized for 5 days before the study (study design is outlined in Fig. 1A.). In part I atherosclerosis was induced in all rabbits by feeding diet A. Six weeks later six rabbits were euthanized (group A). In part II the remaining eighteen rabbits were randomly divided into three groups (B, C and D) and atherosclerosis

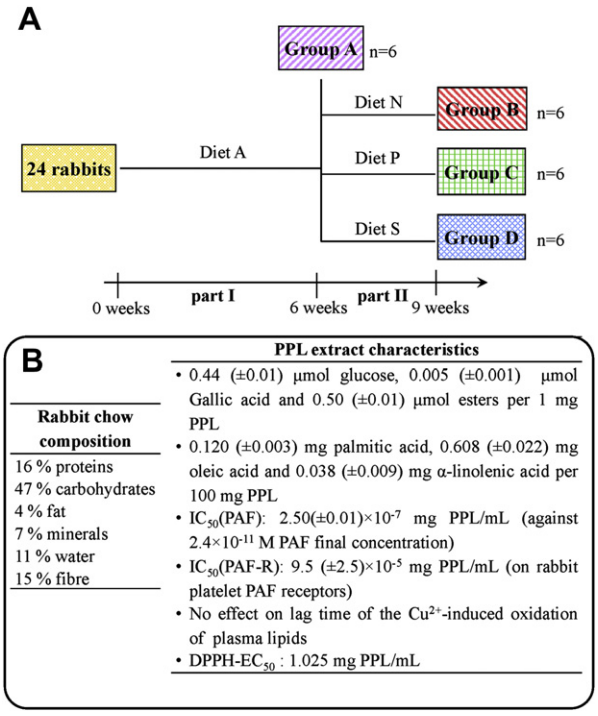

Figure 1 A. Schematic presentation of study design. Twenty-four rabbits were given Diet A for 6 weeks and at the end six rabbits were euthanized. The remaining rabbits were randomly divided into three groups. Each group was given a different diet for 3 weeks and at the end all rabbits were euthanized. Blood was collected at 0, 6 and 9 weeks. Diet A: 99 % rabbit chow + 1% cholesterol (approx. 95% (GC), equivalent to USP/NF, Sigma, St. Louis, MO, USA); Diet N: 100 % rabbit chow; Diet P: 0.34 mg PPL per 1 g rabbit chow (99.966 % rabbit chow + 0.034% PPL); Diet S: 0.13 mg simvastatin (Zocor®, Merck & Co., Inc., NJ, USA) per 1 g rabbit chow (99.987 % rabbit chow + 0.013 % simvastatin). B. Detailed information on rabbit chow composition and PPL characteristics (chemical composition and biological activity).