Long-chain fatty alcohols from pomace olive oil modulate the release of proinflammatory mediators

Angeles Fernández-Archeb, Ana Marquez-Martín, Rocío de la Puerta Vazquezb, Javier S. Peronaa,⁎ Carmen Terencioc, Carmen Perez-Caminoa, Valentina Ruiz-Gutierreza

*Instituto de la Grasa (CSIC), Av. Padre García Tejero no 4, 41012 Seville, Spain
bDepartment of Pharmacology, School of Pharmacy, University of Seville, C/ Profesor García González no 2, 41012 Seville, Spain
cDepartment of Pharmacology, School of Pharmacy, University of Valencia, Av.Vicente Andrés s/n, 46100 Burjassot, Valencia, Spain

Received 28 September 2007; received in revised form 5 January 2008; accepted 11 January 2008

Abstract

Pomace olive oil is a by-product of olive oil extraction that is traditionally produced and consumed in Spain. The nonglyceride matter of this oil is a good source of interesting minor compounds, like long-chain fatty alcohols, which are present free or as part of waxes. In the present study, long-chain fatty alcohols were isolated from the nonglyceride fraction of pomace olive oil, and the composition was identified and quantified. The major components of long-chain fatty alcohols were tetracosanol, hexacosanol and octacosanol. We investigated the ability of long-chain fatty alcohols from pomace olive oil to inhibit the release of different proinflammatory mediators in vitro by cells involved in inflammatory processes. Long-chain fatty alcohols significantly and dose-dependently decreased nitric oxide production by RAW 264.7 murine macrophages stimulated with lipopolysaccharide. Western blot analysis showed that nitric oxide reduction was a consequence of the inhibition of inducible nitric oxide synthetase expression. Long-chain fatty alcohols also reduced tumor necrosis factor-α and prostaglandin E2 production, although the potency of inhibition for the latter was lower. On the other hand, long-chain fatty alcohols significantly reduced thromboxane A2 production in rat peritoneal neutrophils stimulated with the calcium ionophore A-23187. The reduction of eicosanoid release was related to the inhibition of phospholipase A2 enzyme activity by long-chain fatty alcohols, reaching an inhibitory concentration 50% value of 6.2 μg/ml. These results showed that long-chain fatty alcohols may have a protective effect on some mediators involved in the inflammatory damage development, suggesting its potential value as a putative functional component of pomace olive oil.

© 2009 Elsevier Inc. All rights reserved.

Keywords: Pomace olive oil; Long-chain fatty alcohols; Macrophages; Nitric oxide; Inflammation; Cytokine

1. Introduction

Virgin olive oil (VOO), a source of healthy micronutrients, may be regarded as a functional food due to its important beneficial effects. An increasing number of studies are pointing out the important biological effects of some of these micronutrients and that the content of oleic acid cannot fully explain the impact of VOO on health [1,2]. Minor components of VOO, found in the nonglyceric fraction, may also explain some of the healthy effects of the Mediterranean diet [3,4]. Pomace olive oil (POO) is obtained from the wastes after the extraction of VOO by mechanical procedures and contains components from the leaves, skin and seeds. Despite the glyceridic fraction being similar [5], some of the minor components are present in higher concentration in POO than in VOO [6,7]. Thus, the unsaponifiable fraction of POO contains elevated amounts of sterols, tocopherols, waxes and triterpenic acids and alcohols with important biological activities. Among these, recent investigations have revealed the vasorelaxant, antioxidant and anti-inflammatory properties of erythrodiol and oleanolic and maslinic acids [2,8–10].

Another fraction of the minor components of POO that has received insufficient attention to date is that of long-
chain fatty alcohols (LCFAs). These compounds are usually found in waxes of common foods and have been isolated from sugarcane, spinach and beeswax by saponification and solvent extraction. A number of beneficial physiological activities have been attributed to LCFAs, such as reducing platelet aggregation, endothelial damage and cholesterol-lowering effects [11,12]. All these studies have been carried out using “Policosanol”, a commercialized mixture of LCFAs [13,14], whose major components are octacosanol (C28), triacontanol (C30) and hexacosanol (C26). Octacosanol is in fact known for its antiaggregant effect, as an alternative to aspirin for patients suffering from gastric irritation due to its cytoprotective effects [15]. Although present in relatively high concentrations, the biological activities of LCFAs from POO have not yet been studied.

Nitric oxide (NO) has been shown to play a central role in inflammatory and immune reaction activities. Macrophages appear to be the main cellular source of NO since these cells significantly contribute to inducible NO synthetase (iNOS) induction after lipopolysacharide (LPS) incubation [16]. NO is also able to enhance the production of tumor necrosis factor-α (TNF-α) and interleukin 1β cytokines, which participate in the macrophage-dependent inflammation [17]. Activation of macrophages also leads to cyclooxygenase-2 (COX-2) stimulation with consequent prostaglandin E2 (PGE2) overproduction, which plays a key role in the pathogenesis of inflammatory processes [18]. Another eicosanoid of particular importance is thromboxane A2 (TXA2), which is produced by the action of thromboxane synthase on the prostaglandin endoperoxide H2 (PGH2) resulting from the enzymatic transformation of arachidonic acid by the COX-2. TXA2 is a potent inducer of platelet aggregation, vasoconstriction and bronchoconstriction, and has been involved in a series of major pathophysiological conditions [19].

Arachidonic acid is released from phospholipids by the action of the secretory phospholipase A2 (sPLA2), thereby providing the substrate for the biosynthesis of proinflammatory eicosanoids. sPLA2 is released in plasma and other biologic fluids of patients with systemic inflammatory, autoimmune and allergic diseases. Different isoforms of sPLA2 are expressed and released by several cells types, such as neutrophils, basophils, T cells, monocytes, macrophages and mast cells, which promotes cytokine and chemokine production. Thus, sPLA2 might play important roles in the initiation and amplification of inflammatory reaction [20,21]. Therefore, the inhibition of PLA2 could result in down-regulation of the inflammatory response, acting at an early step of several inflammatory mediator biosynthesis.

The aim of our study was to analyze and identify the composition of the LCFAs fraction, isolated from POO, and to examine its ability to inhibit the release of some proinflammatory mediators by cells involved in inflammation. With this purpose, we investigated the effect of LCFAs to inhibit the sPLA2 enzyme activity, in vitro and on NO, PGE2 and TNF-α generation in LPS-stimulated RAW macrophages, as well as the TXA2 produced in rat peritoneal polymorphonuclear neutrophils, stimulated with the calcium ionophore A-23187.

2. Materials and methods

2.1. Sample preparation

The unsaponifiable fraction of POO was isolated following conventional procedures. In brief, POO was saponified with 25 ml of 2 mM KOH in methanol/water (80:20, v/v). After heating at 70°C for 30 min, 50 ml of water and 50 ml of ethyl ether were added. The unsaponifiable fraction was collected by decantation and its components were analyzed following the IUPAC method [22]. Unsaponifiable fraction (1.5 g) was fractionated on a silica gel column and the LCFA subfraction eluted with a mixture of hexane and ethyl ether (85:15, v/v). The same procedure was used to obtain the LCFA fraction that was added to the cell culture medium at appropriate concentrations. For LCFAs analysis by gas chromatography, the isolated LCFAs were transformed into trimethylsilyl ethers by adding 200 μl of a mixture of 9:3:1 v/v/v of pyridine–hexamethyldisilazane–trimethylchlorosilane.

2.2. Gas-chromatography conditions

LCFAs were analyzed with a Chrompack (Middelburg, The Netherlands) CP900 gas chromatograph equipped with a capillary column SGL-5, of 30 m length, 0.25 mm i.d and 0.25 μm film thickness of 5% phenyl methylpolysiloxane stationary phase. Carrier gas was hydrogen, with a head pressure of 110 KPa and 1:60 split ratio. Injector temperature was 280°C and detector temperature 290°C. The oven temperature was programmed from 215°C to 290°C with a rate of 2°C/min. The analysis was carried out following the method of the Commission Regulation (EC) No. 1989/2003; amending Regulation (EC) 2568/91 on the characteristics of olive and the relevant method of analysis. Total LCFAs concentration in POO was calculated as the sum of individual LCFAs concentrations.

2.3. Reagents

[5,6,8,11,12,14,15(τ-3H)] PGE2 was from Amersham Biosciences (Madrid, Spain). [9,10-3H]Oleic acid was purchased from Du Pont, (Itisa, Madrid, Spain). Mouse TNF-α and TXB2 ELISA were from BLK Diagnostics (Badalona, Spain). The rest reagents were purchased from Sigma Aldrich Chem. (St. Louis, MO, USA). Stock solutions of compounds were prepared in DMSO and later dissolved in ethanol. The final concentration of DMSO or ethanol in the culture medium did not significantly influence cell response. The quantities of the compounds are expressed as their final concentration in the culture medium.

2.4. Assay of sPLA₂

Secretory phospholipase A₂ was assayed by using a modification of the method of Franson et al. [23]. *Escherichia coli* strain CECT 101 was seeded in medium containing 1% tryptone, 0.5% NaCl and 0.6% sodium dihydrogen orthophosphate (pH 5.0) and grown for 6–8 h at 37°C in the presence of 5 μCi Ci/ml ³H/oleic acid (sp. Act. 10 Ci/mmol). After centrifugation at 2500×g for 10 min, the cells were washed in buffer (0.7 M Tris–HCl, 10 mM Ca Cl₂, 0.1% BSA, pH 8.0) resuspended in saline and autoclaved for 30–45 min. At least 95% of the radioactivity was incorporated into phospholipids. Human recombinant synovial enzyme was added to a solution of 100 mM (Tris–HCl, 1 mM CaCl₂ buffer, pH 7.5) and preincubated at 37°C for 5 min with 2.5 μl of test compound solution at different doses (1, 5, 10, 50 and 100 μg/ml) or its vehicle in a final volume of 250 μl. Incubation proceeded for 15 min in the presence of 10 μl of autoclaved oleate membranes and was terminated by addition of 100 μl ice-cold solution of 0.25% BSA in saline to a final concentration of 0.07% w/v. After centrifugation at 2500×g for 10 min at 4°C, the radioactivity in the supernatants was determined by liquid scintillation counting. The inhibitory effect was measured in relation to control enzyme activity (radioactivity in cpm) in tubes containing enzyme.

2.5. Cell viability

The mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan was used to assess the possible cytotoxic effect on the mouse macrophage cell line RAW 264.7.

2.6. Culture of murine macrophage RAW 264.7 cell line

The mouse macrophage cell line RAW 264.7 (Cell Collection, Department of Animal Cell Culture, CSIC, Madrid, Spain) was maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with L-glutamine and 10% heat-inactivated fetal bovine serum. Macrophages were removed from the tissue culture flask using a cell scraper and centrifuged at 800×g for 10 min. Cells were resuspended at a concentration of 1×10⁶ cells/ml in a total volume of 200 μl and cultured in 96-well culture plate.

2.7. Nitrite, PGE₂ and TNF-α production in RAW 264.7 macrophages

RAW 264.7 macrophages (1×10⁶ cells/ml) were co-incubated with 10 μg/ml of *E. coli* (Serotype 0111:B₄) LPS at 37°C for 24 h in the presence of LCFAs fraction at different doses (25, 50 or 100 μg/ml) or vehicle. Nitrite (as index of NO generation) was determined in culture supernatants by a flurometric method [24]. PGE₂ and TNF-α levels production were quantified by sandwich immunoassay [25,26].

2.8. Western blot analysis of iNOS expression

To induce iNOS expression, RAW 264.7 cells (2×10⁶ cells/ml) were incubated with LPS (10 μg/ml) in 6-well culture plate in the presence of the highest fraction concentrations (100 μg/ml). Dexamethasone at 10 μM was used as standard reference of iNOS gene expression inhibition. After 18 h of stimulation, medium was removed and cells were washed twice with PBS 25 mM, then cells were lysed with lysis buffer (1% Triton X-100, 1% deoxycholic acid, 20 mM NaCl and 25 mM Tris, pH 7.4). Following centrifugation (10,000×g, 15 min), protein was determined in supernatants by the DC Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA, USA). Equal amounts of protein (25 μg) were loaded on 12% SDS-PAGE and transferred onto pure nitrocellulose membranes for 90 min at 125 mA. Membranes were blocked in PBS–TWEEN 20 containing 3 w/w nonfat milk. Membranes were incubated with specific iNOS polyclonal antibody (1/1500 dilution; Santa Cruz, CA); blots were washed and incubated with peroxidase-conjugated goat antirabbit IgG (1/3500 dilution; DAKO, Denmark). The immunoreactive bands were visualized using enhanced chemiluminescence system (ECL; Amersham Biosciences, Madrid, Spain).

2.9. TXB₂ release by A-23187–stimulated rat neutrophils

Leukocytes containing approximately 85% polymorphonuclear leukocytes and 15% mononuclear cells were prepared [25] from male Wistar rats and resuspended in complete HBSS at 0.5×10⁶ cells/ml containing 1.26 mM Ca²⁺ and 0.9 mM Mg²⁺. Cell viability based on trypan blue exclusion was greater than 95%. Triplicate aliquots of 0.5 ml leukocytes were preincubated at 37°C for 10 min with 2 μl dimethyl sulfoxide (DMSO) containing LCFAs. After this, 5 μl of calcium ionophore A23187 was added to DMSO to give a final concentration of 1 μM for a further 10 min of incubation. The cells were pelleted by centrifugation at 2500×g for 10 min at 4°C, and the supernatants were decanted and frozen. For the TXB₂ assay, aliquots the supernatant were subjected to a commercially available sandwich immunoassay kit.

2.10. Data analysis

The results are presented as mean±S.E.M.; inhibitory concentration 50% (IC₅₀) values were calculated from at least three concentrations. Statistically significant differences were evaluated by analysis of variance (ANOVA) followed by Dunnet’s t test for multiple comparisons.

3. Results

3.1. Analysis of the sample composition

LCFAs were isolated from the POO unsaponifiable fraction, and its constituents were identified by gas chromatography (Fig. 1) as a mixture of very-long-chain
aliphatic alcohols, whose main components were: docosanol (C22) (7.5%), tetracosanol (C24) (30.3%), hexacosanol (37.3%) and octacosanol (15.3%) (Table 1). Additionally, small concentrations of the odd series (n=23, 25, 27) and terpenic alcohols as phytol (C20H40O) or geranylgeraniol (C20H34O) were also detected. Total LCFA concentration in POO was 1853 mg/kg, calculated as the sum of individual LCFA concentrations.

3.2. Inhibition of sPLA2 activity

As shown in Fig. 2, the LCFA fraction was revealed as an effective inhibitor of human recombinant synovial phospholipase A2 activity at all assayed doses (1–100 μg/ml), reaching an IC50 value of 6.2 μg/ml. The percentage of inhibition ranged from about 6.5% at 1 μg/ml to 68% at 100 μg/ml.

3.3. Effect of LCFA on cell viability

LCFAs did not induced toxicity in the macrophage cell line when assessed by mitochondrial reduction of MTT after 24 h of treatment. Viability of cells treated with

| Table 1 Composition of the LCFA fraction from POO analyzed by gas chromatography |
|---------------------------------|------------------|
| Composition (%) | Name          |
| C20H40O           | Phytol         |
| C20H34O           | Geranylgeraniol|
| C22OH             | Docosanol      |
| C23OH             | Tricosanol     |
| C24OH             | Tetracosanol   |
| C25OH             | Pentacosanol   |
| C26OH             | Hexacosanol    |
| C27OH             | Heptacosanol   |
| C28OH             | Octacosanol    |
| C29OH             | Nonacosanol    |
| C30OH             | Triacosanol    |

Fig. 2. Gas chromatogram of the LCFA fraction from POO. Retention times and carbon numbers are shown on the peaks. C21OH: monocosanol; C22OH: docosanol; C23OH: tricosanol; C24OH: tetracosanol; C25OH: pentacosanol; C26OH: hexacosanol; C27OH: heptacosanol; C28OH: monocosanol; C29OH: nonacosanol; C30OH: triacosanol.

**Fig. 1.** Gas chromatogram of the LCFA fraction from POO. Retention times and carbon numbers are shown on the peaks. C21OH: monocosanol; C22OH: docosanol; C23OH: tricosanol; C24OH: tetracosanol; C25OH: pentacosanol; C26OH: hexacosanol; C27OH: heptacosanol; C28OH: monocosanol; C29OH: nonacosanol; C30OH: triacosanol.

Fig. 2. Effect of LCFA on human recombinant synovial phospholipase A2 activity. Results show means±S.E.M. from three different experiments (*P<.05 and **P<.01 vs. control). Control: total PLA2 enzyme activity. The LCFA fraction was extracted from POO as described in the Materials and methods section.
LCFAs was 87±0.8%, 95±0.8% and 99±0.5% at LCFAs concentrations 100, 50 and 25 μg/ml, respectively.

**4. Nitrite, PGE\(_2\) and TNF-α production by LPS-stimulated RAW 264.7 macrophages**

The effect of LCFAs on the release of inflammatory mediators by LPS-stimulated RAW 264.7 macrophages is depicted in Fig. 3. Co-incubation with LCFAs significantly reduced nitrite production, in a dose-dependent manner, with an IC\(_{50}\) value of 53.4 μg/ml (Fig. 3A). The highest inhibition was achieved at 100 μg/ml (88%), but even at the lowest concentration assayed (1 μg/ml), an inhibition of 20% was observed.

Likewise, PGE\(_2\) generation was also reduced by LCFAs at all doses assayed, although the percentage of inhibition was lower than that of nitrite (Fig. 3B). The highest inhibition was found at 100 μg/ml and was quantified as 38% of total PGE\(_2\) production.

In contrast, LCFAs were able to significantly inhibit TNF-α generation only at the highest dose assayed (100 μg/ml) (Fig. 3C). Nevertheless, the percentage of inhibition was very high (83%).

**3.5. iNOS expression**

Western blot assay in LPS-stimulated RAW 264.7 macrophages was performed to assess possible effects on iNOS enzyme gene expression. Fig. 4 shows that LCFAs at 100 μg/ml caused a complete inhibition of the LPS-induced iNOS expression (100%). This effect was similar to dexamethasone, the reference compound.

**6. Effect of LCFAs on the TXB\(_2\) release by rat neutrophils**

LCFAs inhibited TXA\(_2\) generation (measured as TXB\(_2\)) release by rat neutrophils stimulated with calcium ionophore A23187. Each value represents the mean±S.E.M. for three triplicate experiments. **P<.01; ***P<.001 vs. LPS control group. The LCFAs fraction was extracted from POO as described in the Materials and methods section.
A.23187 at all assayed doses (Fig. 5). The concentration-dependent study showed an IC$_{50}$ value of 56.17 μg/ml.

4. Discussion

POO is a by-product of VOO extraction, obtained from the wastes originated after the first pressing of the olive fruit. The new improved procedure for POO extraction by centrifugation, instead of using organic solvents, allows the presence of higher concentrations of interesting minor components from the olive fruit and leaves. The present study demonstrates that a fraction isolated from POO, constituted by LCFAs, is able to reduce the generation of mediators involved in the inflammatory response in different experimental models.

Chemical and nutritional studies have shown that mixtures of long-chain primary alcohols extracted from waxy materials from different sources, such as beeswax, rice bran, wheat germ, sugar cane and grain sorghum, are able to exert several beneficial physiological effects. The main linear aliphatic alcohols present in olive oil are tetracosanol (C24), hexacosanol (C26), octacosanol (C28) and docosanol (C22) and are found in significantly larger amounts in POO than in VOO [27]. In agreement with these authors, the main fatty alcohol present in the LCFA fraction isolated from POO was hexacosanol (37.3%), followed by tetracosanol (30.3%), octacosanol (15.3%) and docosanol (7.5%), accounting for 90% of total LCFA. This composition is slightly different of that of policosanol, a commercial mixture of very-long-chain alcohols composed mainly of octacosanol (~60%), triacontanol (~13%) and hexacosanol (~6%) [12]. A number of clinical studies have documented the ability of policosanol to reduce serum cholesterol [11,14,28], although a very recent clinical study was unable to find a significant cholesterol-lowering effect [29]. Other studies had demonstrated that this alcohol mixture, administrated orally to rabbits, was effective in preventing the development of atherosclerosis lesions in the aorta [11]. Atherosclerosis is considered a low-grade chronic inflammatory disease as it shows many of the hallmarks of an inflammatory disease [30]. Many studies have shown that the process of atherogenesis in part starts with the interaction between activated leucocytes and activated endothelium [31]. Molecules including cytokines, chemokines, growth factors and prostanoids have an important role in vascular homeostasis.

Macrophages play a key role in the development of the atherosclerotic lesion [32]. The onset of atherosclerosis involves attraction of monocytes to the vessel wall at local sites of endothelial dysfunction. The monocytes subsequently migrate through the endothelium and differentiate into macrophages. Atherogenesis is accompanied by the local production and secretion of inflammatory mediators, for which the macrophage is a major source. Activated macrophages secrete eicosanoids, nitric oxide and proinflammatory cytokines, which play a key role in the pathogenesis of the inflammatory disease. Thus, the expression and activity of iNOS and COX-2 greatly modulate the synthesis of NO and prostaglandins and, hence, the inflammatory response. Shinbori et al. [33] have recently reported that hexacosanol (C26) can improve diabetes-induced hypercontraction by down-regulation of iNOS expression. Our study demonstrates that LCFAs are able to inhibit nitrite production from LPS-stimulated RAW 264.7 macrophages culture without indication of cytotoxicity. The reduction of nitrite concentrations in supernatants of cells co-incubated with LPS and the fraction tested can be either due to an interference with the enzyme induction by LPS or due to a direct action of LCFAs on iNOS activity. Western blot analysis showed that the LCFAs fraction is highly involved in iNOS protein expression. Thus, this fraction may selectively inhibit the enhanced iNOS expression and might control high production of NO during inflammatory or immune conditions. Statins are potent hypcholesterolemic drugs, which have been shown to suppress iNOS gene expression in RAW 264.7 macrophages [34]. Like statins, LCFAs are also hydroxymethyl-glutaryl-coenzyme A reductase inhibitors and, in fact, the cholesterol-lowering effect of policosanol has been attributed to this activity [12]. For this reason, it has been hypothesized that LCFAs might mimic the effect of statins on iNOS expression [35]. Should this hypothesis turns out to be correct, LCFAs might modulate NO release by acting on iNOS transcription, probably by inactivating the nuclear transcription factor NFκB, just as statins do [36].

LCFAs also reduced TNF-α production by LPS-stimulated RAW 264.7 macrophages but only at the highest concentration tested (100 μg/ml). Hexacosanol has also been shown to decrease cytokine concentrations in the kidney of streptozotocin-induced diabetic rats [37]. In fact, Muller et al. [38] reported that synthetic LCFAs, bearing an α-tocopherol moiety, reduce TNF-α secretion by LPS-activated microglia. According to these authors, this effect would be also mediated by NFκB activation. This transcription factor is also involved in COX-2 transcription [39], the enzyme responsible for the synthesis of PGE$_2$. Our results show that LCFAs also reduce PGE$_2$ release in a dose-dependent manner, although the effect was not so potent. Therefore, our results overall suggest that the effect of the LCFAs fraction from POO might be mediated by the inactivation of NFκB.

As well as promoting cytokine and chemokine production by macrophages, sPLA$_2$ releases arachidonic acid from phospholipids, providing the substrate for the biosynthesis of PGE$_2$ by COX-2. Therefore, sPLA$_2$ plays an important pathophysiological role in the initiation and amplification of the inflammatory reaction [20]. LCFAs isolated from POO revealed as potent inhibitors (IC$_{50}$: 6.2 μg/ml) of human recombinant sPLA$_2$ in vitro. IC$_{50}$ or the half maximal inhibitory concentration, represents the concentration of particular molecule that is required to inhibit some biological processes by 50% and is used as a measure of this molecule.
potency; therefore, the smaller IC$_{50}$ the less molecule amount needed to exert a biological process. In our study, only 6.2 µg/ml of LCFAs was needed to inhibit PLA$_2$ production by 50%, which shows the high potency of LCFAs. The reduction of de PLA$_2$ enzymatic activity was even significant at the lowest dose assayed (1 µg/ml). These promising results, in the experimental model used, suggest that LCFAs fraction could have a possible role as functional component of POO.

Neutrophils migrate to the site of inflammation and, upon activation by different stimuli, generate large amounts of proinflammatory mediators including TXA$_2$, an arachidonic-derived bioactive metabolite with particular importance in the pathogenesis of inflammatory and thromboembolic diseases. TXA$_2$ is produced by the action of thromboxane synthase on the PGH$_2$, which results from the enzymatic transformation of arachidonic acid by COX. It is a potent inducer of platelet aggregation, vasoconstriction and bronchoconstriction, and has been involved in a series of major pathophysiological conditions [40]. LCFAs significantly reduced TXA$_2$ (measured as TXB$_2$) in a dose-dependent manner after overproduction by calcium ionophore A23187-stimulated neutrophils. These results agree with others experimental studies carried out with policosanol. This mixture of LCFAs has been reported to lower TXA$_2$ serum levels in both animals and humans, which was accompanied by arachidonic acid-induced platelet aggregation [14,41].

A number of very recent investigations are pointing out that POO or some its components may have potent activities against the development of atherosclerosis [2,8,9,42]. Our study demonstrated that the LCFAs fraction, isolated from POO, can reduce the release of different inflammatory mediators (eicosanoids, cytokines and NO) by interfering in different stages of their metabolic pathways, like iNOS expression or PLA$_2$ activity. Thus, the present results suggest that the LCFAs fraction may confer a protective role to POO against inflammatory damage in different pathologies, including atherosclerosis.

Acknowledgments

This study was supported by funds from “Comision Interministerial de Ciencia y Tecnología (CICYT AGL2002-00195 and AGL2005-00572).

References


