Chapter

VIRGIN OLIVE OIL AS A SOURCE OF ANTI-INFLAMMATORY AGENTS

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ABSTRACT

Virgin olive oil (VOO) has many potential health benefits, including the amelioration of inflammatory processes. In part, this is known to occur through the modification of the endothelial function, leading to a decrease of the levels of cell-adhesion molecules (CAMs), including the inter-cellular adhesion molecule 1 (ICAM-1) and the vascular cell adhesion molecule 1 (VCAM-1). Importantly, virgin olive oil is able to inhibit the tumor necrosis factor-alpha (TNF-α), that is a key cytokine in controlling distinct types of cell functions and a particular therapeutic target for inflammatory diseases. Moreover, *in vitro* and *in vivo* assays with virgin olive oil or its main components clearly indicate a marked modulation of signaling pathways regulating the activation of pro-inflammatory mediators, including the nuclear transcriptional factor NF-κβ, the cytokines interleukin-1 (IL-1), and interleukin-6 (IL-6), and the enzymes cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX) and inducible nitric oxide synthase (iNOS). So far, the cellular and molecular anti-inflammatory mechanisms of virgin olive oil have been particular associated with its high amounts of phenolic compounds, as well as to its composition in mono and polyunsaturated fatty acids. Still, the available data is disperse and needs consolidation, in order to allow solid conclusions on this issue. The present chapter summarizes the epidemiological data and intervention trials focusing the effects of virgin olive oil in inflammatory processes and/or inflammatory related-

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diseases, as well as the main virgin olive oil constituents associated to the protection process and their underlying mechanisms of action.

Keywords: Virgin olive oil; inflammation; atherosclerosis; bowel diseases; cancer; hydroxytyrosol; oleic acid; oleuropein; unsaponifiable fraction

INTRODUCTION

Mediterranean diet has been associated to the prevention of distinct diseases such as coronary diseases, bowel ailments, cancer and aging [1-3]. Olive oil is one of the prime ingredients of the Mediterranean diet, thus playing an important contribution for its beneficial properties. In particular, the latter have been closely associated to VOO, i.e., the oil resultant only from the pressing of olives [4].

The main beneficial property attributed to VOO is undoubtedly its capacity to prevent cardiovascular diseases. The U.S. Food and Drug Administration (FDA) recognized that, due to its high content in monounsaturated fatty acids (MUFAs, in particular oleic acid C18:1), the daily ingestion of approximately two tablespoons (23 g) of VOO exerts benefic effects on the risk of coronary heart disease [5]. However, supposing that these beneficial effects would only be due to the oil´s MUFAs contents, then any type of oleic acid-enriched oils (such as rapeseed oil) or any MUFAs-rich food would have similar health benefits. As this is not observed, it was assumed that other VOO's components must also contribute to its beneficial effects [6].

It has been suggested that the VOO´s minor components, in particular the phenolic compounds, are as well key agents contributing for its health benefits. These are also believed to exert synergistic effects with MUFAs [7]. Besides those, triterpenic dialcohols, phytosterols, tocopherols, hydrocarbons and volatile and aromatic compounds are equally important in establishing the VOO´s bioactive properties [6]. The present chapter summarizes the main claimed anti-inflammatory properties of VOO´s, as well as the individual constituents associated to this property.

1. Inflammatory Process

The inflammation is a body defense mechanism whose goal is to eliminate the injury caused by pathogens or by the action of physical agents. Overall, the inflammatory process comprises two interconnected defense mechanisms, i.e., an unspecific response (innate immunity) and a highly specific one (adaptive immunity) [8]. The cells of the innate system residing in tissues (e.g. macrophages, fibroblasts, mast cells and dendritic cells) as well as circulating cells (e.g. monocytes and neutrophils) express pattern recognition receptors (PRRs), i.e. proteins able to directly or indirectly recognize pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) that are released by injured cells [9]. When activated, PRRs form multi-subunit oligomeric complexes which then trigger signaling cascades that help to contain the infection and the activation of the adaptive immune response [10]. In turn, the activation of this immune system causes the increment of

microbial-specific leukocytes (e.g. T and B cells), a process that is highly effective and specific but that takes days to fully develop [10].

Hence, overall, the common hallmarks of inflammation, i.e. swelling, redness, pain and heat are primarily initiated by the innate response. Notably, the cells of the adaptive immune response can contribute to and exacerbate these effects, but those of the innate immune system are also strictly associated to the inflammation terminus process [11].
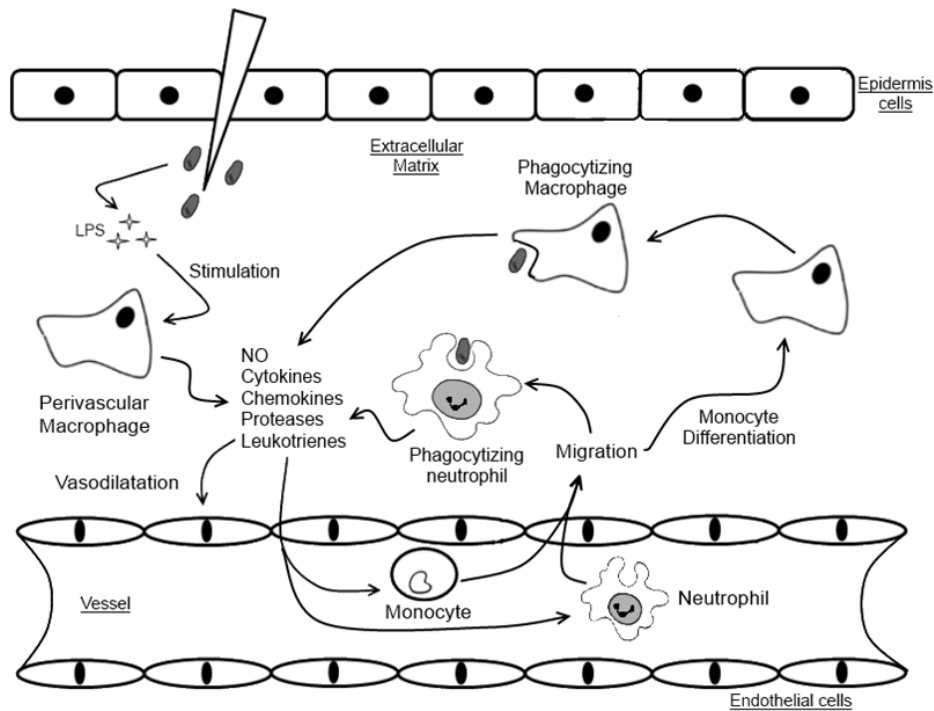

Figure 1. Cells and mediators of the innate inflammatory response. The injury or the pathogenic stimuli activate the perivascular macrophages around the injured area. These begin to phagocyte the infectious agents and release inflammatory mediators to the blood that will increase the permeability of the vessel and recruit more neutrophils and monocytes. The leukocytes on the blood cross the vessel and migrate to the injured area, where the monocytes differentiate into macrophages and, together with neutrophils, they will phagocyte the pathogenic agents and produce more inflammatory mediators until the clearance of the pathogenic.

Inflammation usually begins in a localized area, although depending on the injury´s severity, it can quickly become systemic. The acute phase of inflammation starts immediately upon injury (see Figure 1) and rapidly turns severe but notably, it persists only for a short period and is usually beneficial to the host [12]. This is mainly characterized by the rapid increase in blood flow to the affected region, which is supplied by the dilated arterioles. The capillaries also become more permeable, so that fluid and blood proteins move into the interstitial spaces, together with neutrophils and possibly some macrophages. Note that neutrophils are the most abundant leukocytes in the blood and they represent a first line of attack of the immune system. Likewise macrophages, they are capable of emitting phagocytes cytoplasmic processes involving foreign particles through the digesting enzymes present in cells (Figure 1) [13]. Overall, these events allow the removal of the noxious stimulus via

phagocytosis, which is then followed by the resolution of the inflammation. Sore throat from a cold or flu, a cut on the skin or a blow are typical conditions that can result in acute inflammation [14].

On the other hand, if the inflammation lasts for a long period of time, a second stage (or chronic inflammation) is settled and may predispose the host to various chronic inflammatory illnesses. The beginning of this type of inflammation is characterized by the replacement of neutrophils by macrophages and other cells, including the T cells (also known as T lymphocytes i.e., a sub-class of leukocytes that plays a key role in the regulation of the immune system, in particular the adaptive) [15]. The evolution of this condition is also histologically associated with the proliferation of blood vessels, fibrosis and necrosis. Typical examples of chronic inflammation conditions include, in between many, asthma, tuberculosis, rheumatoid arthritis and lupus [12].

Notably, vascular and cellular reactions of acute and chronic inflammation are mediated by many chemical mediators (proteins or plasma cells) which are produced and/or released by the cells of the immune system. Once activated and/or released by the cells, most of these mediators have a short life and exert their biological activity through binding to specific receptors on target cells, although others possess enzymatic activity (lysosomal proteases). Note that a mediator may itself stimulate the release of other mediators by target cells. The most relevant inflammatory mediators are further detailed in below [16].

1.1. Chemical Mediators

Inflammatory mediators can be generally grouped with respect to their origin: plasma or cellular. While the former are present in plasma in the form of precursors and suffer activation through a number of proteolytic cleavages, the cellular mediators are usually preformed and further stored in intracellular granules (e.g. histamine) or alternatively, synthesized *de novo* (e.g. prostaglandins and cytokines) [17].

The two vasoactive amines histamine and serotonin, together with lysosomal enzymes, comprise the most relevant preformed cellular mediators. These mediators are stored in secretory granules and are involved in the initial stage of the inflammatory process [18]. In particular, histamine is synthesized by basophils (a subtype of granulocytes leukocytes), platelets, and especially by mast cells (resident cells of several types of tissues) and this mediator acts by interacting with three distinct receptors present on target cells, known as H_1, H_2 and H_3 [19]. From those, the H_1 receptor causes contraction of bronchial smooth muscle, intestine and uterus and increases the permeability of venous capillaries. In turn, serotonin is mainly found in the lining of the gut, in platelets and in central nervous system. Such as histamine, serotonin is a vasodilator and increases vascular permeability [19]. Its release from the platelets is triggered by platelet aggregation, upon contact with collagen, thrombin, adenosine diphosphate and antigen-antibody complex [14]. In turn, as previously mentioned, lysosomal enzymes are released by macrophages and neutrophils with the direct purpose of destroying the pathogenic agents [14].

From the newly synthesized inflammatory mediators, arachidonic acid (AA) and other polyunsaturated fatty acids (PUFAs) plus their products deserve special mention in view of their role in inflammation, resolution of inflammation, and inhibition of production of proinflammatory cytokines [20]. Arachidonic acid is a polyunsaturated acid present in phospholipids of the plasma membrane (particularly in phosphatidylcholine,

phosphatidylethanolamine and phosphatidylinositides) that can be enzymatically released by the action of enzyme such as phospholipase A2 (PLA2) [21].

The AA is subsequently transformed through cyclooxygenase (COX) and lipoxygenase (LOX) pathways to various biologically active metabolites (e.g. prostaglandins, thromboxane, leukotrienes and lipoxins) collectively termed as eicosanoids [22]. Note that eicosanoid production is considerably increased during inflammation and both COX and LOX pathways are of clinical relevance. Particular interest has been given to the isoenzyme COX-2 [23] that is primarily expressed at sites of inflammation and produces pro-inflammatory eicosanoids and to 5-LOX, which is the key enzyme in leukotriene biosynthesis (considered as potent mediators locally released at the inflammation site by leukocytes and other 5-LOX expressing cells) [24].

Cytokines, a diverse group of proteic mediators (anti-inflammatory and pro-inflammatory), are also determinant in the inflammatory response [25]. Any disorder in the regulation of cytokines can lead to the development of inflammatory diseases [26]. Tumor necrosis factor-α (TNF-α) is one of the most important inflammatory cytokines that controls different types of cell functions [27]. This is produced in its active form mainly by macrophages, but also by other immune cells including mast cells, neutrophyls, T cells and natural killer cells (NK, i.e, effector lymphocytes of the innate immune system that limit the spread of tumors or microbial infections). At the cellular level, TNF-α is a potent activator of neutrophils, mediating adhesion, cellular degranulation and chemotaxis, as well as interacting with the endothelial cells to induce the appearance of adhesion molecules such as the intercellular adhesion molecule-1 (ICAM-1), the vascular cell adhesions molecule-1 (VCAM-1) and E-selectin [28].

The pro-inflammatory effects of TNF-α are mainly due of its ability to activate nuclear factor kappa B (NF-κB) [29], through a series of complex signaling cascades leading to the degradation of the IκBκ (an inhibitor of NF-κB activation). When activated, the NF-κB translocates to the nucleus, binds to the promoter or enhancer regions of target genes to enhance transcription [26]. There are many genes known to be regulated by NF-κB, including TNF-α itself and others such as COX-2, 5-LOX, cell-adhesion molecules (CAMs), inflammatory cytokines and inducible nitric oxide synthase (iNOS). Due to its central role in inflammation process, this pathway is a particular therapeutic target [26].

Besides the activation of NF-κB, TNF-α is also capable of activating the mitogen-activated protein kinases (MAPKs) pathway, a class of proteins that are involved in the regulation and/or activation of a series of transcription factors. In particular the P38 MAPKs are involved in cell differentiation, apoptosis and autophagy [30].

Interleukins (IL) are also a central group of cytokines. These are primarily produced by T helper cells (T_H or CD4 lymphocytes i.e, a specific population of T cells), but also by monocytes, macrophages and endothelial cells. They mainly induce the development and differentiation of T cells as well as of B cells (a lymphocyte population whose main function is the production of antibodies), fibroblasts and endothelial cells [9]. Each IL acts on a limited set of cells which express the appropriate receptors [31]. The best characterized pro-inflammatory interleukins are the IL-1, IL-2, IL-6 and IL-8, while IL-4 and IL-10 are two major anti-inflammatory ILs.

Interforon gama (IFN-γ) is another key cytokine, that is mainly produced by T cells, B and NK cells. Its main function is the macrophage activation, rendering them able to exert its microbicidal functions [32]. In addition, it also promotes the differentiation of T helper

lymphocytes to the Th1 subpopulation (the host immunity effectors against intracellular bacteria and protozoa). IFN-γ induces the transcription of many genes in macrophages, including those for the production of antimicrobial molecules such as oxygen free radicals and nitric oxide, which represent one of the best effector mechanisms for elimination [32].

Note that superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), inducible nitric oxide (iNO), and other reactive oxygen species (ROS) are indeed key mediators with an important role in vascular and cellular components of inflammatory reactions [14]. These are produced not only by macrophages, but also by other immune cells including activated neutrophils and monocytes, T-cells, Kupffer cells and glial cells [33].

2. Targets of inflammation by VOO

2.1. Anti-Inflammatory General Mechanisms

Several studies report the fact that VOO and/or its components hamper numerous inflammatory processes. In fact, Eisner et al. [34] observed that leukocytes collected from VOO-intestinally treated rats were less susceptible to lipopolysaccharide (LPS).

From all the VOO´s components, the phenolics, and in particular hydroxytyrosol, are the main compounds associated to the oil´s anti-inflammatory properties. Recently, Pontoniere [35] referred that the VOO´s phenolic fraction was able to block the activation of NF-κB and consequently, the expression of cytokines, of LOX and COX, also affecting the production of adhesion molecules and eicosanoids derived from arachidonic acid. In turn, studies performed in LPS-stimulated murine macrophages (RAW 264.7 cells) treated with hydroxytyrosol have demonstrated that this phenolic is able to attenuate iNOS and COX-2, and to decrease the secretion of prostaglandin E_2, plus of several pro-inflammatory citokynes (TNF-α, IL-1 α and β, IL-6 and IL-12) and chemokines (a family of small cytokines), as well as to reduce the gene expression of the metalloproteinase-9 (MMP-9, a protease of the MMP´s family, which are closely associated to the inflammatory process) [36-38]. Moreover, it was also demonstrated that this phenolic compound exerted a negative effect on NF-κB pathway [38, 39]. Similar results were obtained in phorbol 12-myristate 13-acetate (PMA)-stimulated human monocytes. Indeed, in this cellular model, hydroxytyrosol was able to decrease the transcription of iNOS and TNF-α, the COX-2 expression and the production of superoxide ion and of prostaglandin PEG_2 [40, 41].

Besides hydroxytyrosol, oleuropein and/or oleuropein glucoside plus other VOO´s phenolics were also reported to counteract inflammatory events. In this sense, Visioli et al. [36] demonstrated that hydroxytyrosol and oleuropein showed great biological activities, including the ability to inhibit platelet aggregation, as well as to scavenge hypochlorous acid, superoxide ion and other ROS, overall resulting in an increased plasma antioxidant capacity. Notably, the authors concluded that these compounds were more effective than butylated hydroxytoluene (BHT), which is a potent synthetic antioxidant. Also, De la Puerta et al. [42] reported that hydroxytyrosol, oleuropein, caffeic acid, and tyrosol could inhibit 5-LOX activity, thus diminishing the leukotriene B_4 production on rat peritoneal leukocytes, while Miles et al. [43] reported that oleuropein glycoside, caffeic acid and kaempferol caused a high inhibition on the IL-1β and PEG_2 production levels, on human blood cultures. Furthermore, as demonstrated by Dell´Agli et al. [44], oleuropein is also an intervening phenolic on the

reduced expression and secretion of MMP-9, as demonstrated in TNF-α-stimulated monocyte (THP-1) cells. The authors showed that this effect was due to the impairing effect of oleuropein on the NF-κB signaling pathway [44].

Additionally, oleocanthal (another VOO´s potent antioxidant) is being a target of interest due to its strong anti-inflammatory properties. This phenol has been proved to have strong inhibitory effects on the prostaglandin biosynthesis by hampering the activity of COX-1 and COX-2, mimicking the anti-inflammatory effects of the well-known drug ibuprofen. Even more, for equimolar concentrations, oleocanthal exhibited greater inhibitory effects on COX than ibuprofen [9, 45].

Recently, the work of Cardeno et al. [46] has demonstrated, for the first time, the anti-inflammatory and antioxidant properties of the VOO´s unsaponifiable fraction, on a LPS-stimulated murine macrophages model. In particular, the authors showed that this fraction exerted a strong inhibitory ability on intracellular ROS and NO production. This also decreased the expression of COX-2 and iNOS and, more importantly, it induced down-regulation of the NF-κB signaling and MAPK phosphorylation pathways.

Additional studies also reported the anti-inflammatory effects of some individual unsaponifiable components. One of those components is α-tocopherol, which has been shown to inhibit the expression of 5-LOX, COX-2 and IL-1β [37, 47, 48]. Furthermore, β-sitosterol, the main VOO's phytosterol, was shown to influence the reduction of ROS production and arachidonic acid release, as well as COX-2 activity and PGE2 production in phorbol ester-induced macrophages [49]. These results are in agreement to those of Moreno et al. [50], whom verified that ROS and NO production, arachidonic acid release and arachidonic acid metabolites synthesis (through the COX and LOX pathways) were impaired in PMA-stimulated macrophages RAW 264.7, when exposed to β-sitosterol and two other minor components of VOO, i.e, squalene and tyrosol.

2.2. Atherosclerosis

Atherosclerosis is the main inflammatory disease in which VOO is claimed to exert beneficial effects. Inflammation in atherosclerosis is due to injury or change of endothelium function that might be triggered by several causes (step 1 in Figure 2), including an excess of reactive oxygen species, or the exposure to toxic agents (e.g. oxidized low density lipoprotein cholesterol, oxLDL), to infectious agents or advanced glycosylated end products (the result of an oxidation reaction with glucose that results in a type of oxidant commonly found in the blood of diabetics) [51, 52].

The inflamed endothelium then expresses selective adhesion molecules, namely the VCAM-1 and ICAM-1 respectively (step 2 in Figure 2). As a result, and in opposition to the normal epithelium, the inflamed one is able to bind various classes of leukocytes (step 3 in Figure 2), which in turn are exponentially recruited by the increased expression of specific cytokines and of their pro-inflammatory downstream events, e.g. NF-κB, COX and LOX (steps 4 and 5 in Figure 2). Increased endothelial stress also stimulates the arterial smooth muscle cells to produce proteoglycans that are able to bind and retain lipoprotein particles and increase their oxidative modification, also contributing for the reinforcement of the inflammatory process [53].
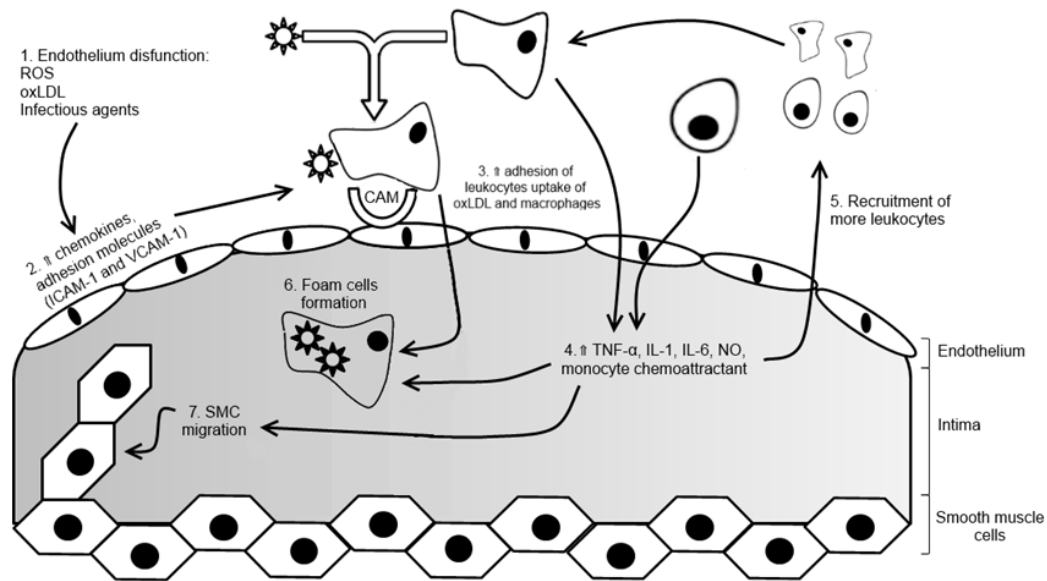
Figure 2. Endothelium dysfunction and atheroma formation. Factors as reactive oxygen species (ROS), oxidized LDL cholesterol (oxLDL) or other infectious agents can promote endothelium dysfunction. The production of cellular adhesion molecules (CAM) allow the binding of blood circulating leukocytes, which in turn will migrate into the intima and further release inflammatory mediators. The macrophages start to phagocyte the oxLDL particles, thus forming foam cells and, at the same time, more leukocytes are recruited to the intima, thus amplifying the inflammatory mediators. As the inflammation and the deposition of foam cells grow, the smooth muscle cells start to migrate to form a coat around the injured area.

In particular, monocytes differentiate into macrophages and express the scavenging receptors, allowing them to bind and engulf these modified lipoproteins, leading to the formation of lipid-laden macrophages i.e. foam cells (step 6 in Figure 2). Macrophages and other leukocytes also release cytokines and grown factors that are central for stimulating the migration and proliferation of smooth muscular cells (step 7 in Figure 2), ending up with the formation of a typical dense extracellular matrix [54].

Besides its crucial role in initiation and establishment of atheroma, one should highlight that inflammatory processes are also decisive in the acute thrombotic complications of atheroma, as the combined action of macrophages and lymphocytes contribute for the production of proteinases such as MMP-9, which degrade extracellular matrix proteins including collagen and elastin and hence cause the narrowing of the fibrous cap, rendering it susceptible to rupture [53].

On the other hand, literature data support the fact that VOO and/or its components hamper numerous atherosclerosis-related inflammatory processes. In particular, Camargo et al. [55] reported that a Mediterranean diet enriched in VOO can indirectly inhibit the expression of NF-κB and that of metalloproteinase MMP-9. Moreover, in high/medium-risk cardiovascular disease patients, the administration of VOO has resulted in a reduction of white blood cells, as well as of the endothelium ICAM expression, pointing for an amelioration of the endothelium function [56]. Additionally, Urpi-Sarda et al. [57] reported that a Mediterranean diet enriched in VOO promoted the reduction of IL-6 and ICAM

molecules on plasma of high-risk cardiovascular disease patients. The IL-6 reduction effect of VOO has also been shown by Fitó et al. [58], in a similar test model.

According to literature data, health-promoting VOO´s components in atherosclerosis-related inflammatory processes mainly enclose oleic acid, as well as some phenolics and terpenes. Oleic acid is very well known for its ability to reduce the blood levels of ROS, LDL and its oxidative modification into oxLDL cholesterol (Figure 3) [59-61]. Moreover, the consumption of oleic acid by Man has been described to increase the levels of HDL cholesterol and to protect it against oxidation [62]. Note that while ROS and oxLDL cholesterol are known to increase the risk of atherosclerosis, elevated HDL cholesterol levels are believed to ameliorate the lipid efflux from the foam cells to the HDL and hence, to counteract the inflammatory atherosclerotic process [4] (Figure 3). HDL particles can also transport antioxidant enzymes that can break down oxidized lipids and neutralize their pro-inflammatory effects [53].

Furthermore, incorporation of oleic acid in total cell lipids of an *in vitro* stearic acid-induced model of early atherogenesis caused the decrease on the incorporation of this fat acid in the phospholipids, as well as a decrement of NF-κB activation, thereby down-regulating the expression of several endothelial and leukocyte adhesion molecules, among which the VCAM-1 and ICAM-1 (represented in Figure 3) [63, 64]. This was reinforced by the work of Sanadgol et al. [65] which reported the suppressed expression of the two CAMs on LPS-stimulated human bone marrow endothelial cells treated with oleic acid.
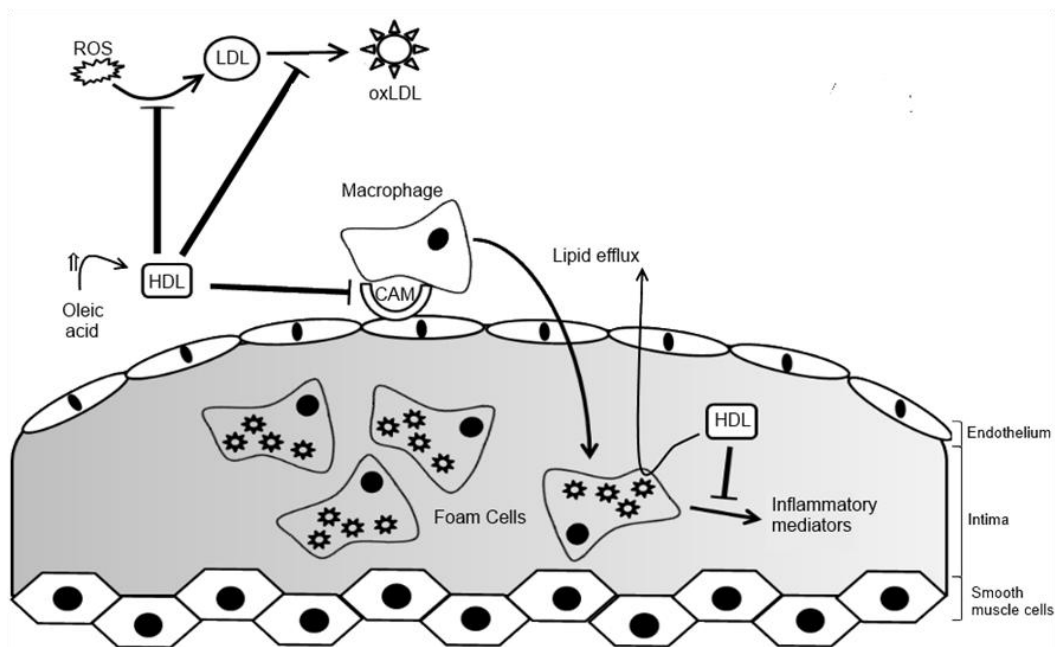

Figure 3. Influence of oleic acid and HDL cholesterol on the atheroma formation. Oleic acid promotes the increase of HDL cholesterol levels, which in turn interfere with reactive oxygen species (ROS) and block the oxidation of the LDL into oxLDL. HDL cholesterol also impairs the adhesion of the leukocytes to the endothelium, preventing their accumulation in the atheroma. Moreover, it can inhibit the release of inflammatory mediators and interact with the foam cells, by promoting the lipid efflux from the cells to the bloodstream and hence ameliorate the inflammatory condition.

Regarding the protective effects of polyphenols, Covas et al. [66] have shown that the intake of polyphenols-enriched VOO decreased the levels of LDL cholesterol and it simultaneously increased those of HDL cholesterol. Moreover, a similar study reported an increased up-regulation of several lipid efflux related genes including peroxisome proliferator activated receptors (PPARs)(α, γ and δ), ATP-binding cassette transporter-A1 (ABCA1) and COX-1 [62]. As a consequence of the activation of PPARs, the authors have described an increase on the HDL cholesterol levels and a down-regulation of CD40-ligand expression and of its related products, such as vascular endothelial growth factor, ICAM-1 and IFN-γ [67, 68].

Amongst the VOO´s phenolic constituents, hydroxytyrosol is by far the most associated to its claimed protective effects on atherosclerosis. In more detail, Zrelli et al. [69] described that hydroxytyrosol was able to induce an indirect dose-dependent inhibition of the NF-κB on vascular endothelium cells. Furthermore, this polyphenol was shown to inhibit MMP-9 release, an effect that was potentiated in the presence of oleic acid [7].

Notwithstanding, hydroxytyrosol is not the only polyphenol capable of inhibiting MMP-9, as oleuropein was also observed to have similar preventive effects on this metalloproteinase [3]. Besides, it was also reported that hydroxytyrosol has the ability to restrain platelet aggregation and the synthesis of eicosanoids in human blood, as well as the reduction of the expression of VCAM-1 in endothelial cells [70, 71].

The potent antioxidant activity of hydroxytyrosol was also shown to prevent increasing levels of ROS formation in pulmonary artery endothelial cells in the presence of H_2O_2 [72]. This ROS preventing ability was reinforced by Scoditti et al. [3] in a study showing that hydroxytyrosol and oleuropein caused the decrease of the intracellular levels of ROS formation, as well as a reduction of the activation of NF-κB, in PMA-stimulated human umbilical vein endothelial cells (HUVEC). The authors also tested the ability of these polyphenols to counteract the expression of COX-2 in the same HUVEC model, concluding that both hydroxytyrosol and oleuropein were able to cause a decrease of 50% on the expression of this enzyme, without affecting the constitutive expression of COX-1.

Another study focusing the effects of oleuropein, hydroxytyrosol and homovanillyl alcohol on HUVEC surface and on the mRNA expression of three adhesion molecules (ICAM-1, VCAM-1 and E-selectin) concluded that the two first polyphenols induced a decrement on the ICAM-1 and VCAM-1 expression (surface and mRNA), while homovanillyl alcohol caused a reduction on the cell surface expression of the three adhesion molecules, although no effect was observed for their corresponding mRNA levels [73].

Moreover, expression of surface scavenger receptors on LDL-stimulated macrophages was shown to be reduced in the presence of the phenols tyrosol and hydroxytyrosol and of the hydrocarbon squalene, thus lowering the macrophages lipid intake [74]. Moreover, experimental tests performed on rabbits submitted to an atherosclerotic diet against diets with squalene or hydroxytyrosol were compared, revealing that the polyphenol reduced the endothelium inflammatory activation while the hydrocarbon decreased the fibrosis [75].

The unsaponifiable fraction of the VOO is also rich in antioxidant and anti-inflammatory compounds. α-Tocopherol has a great potential as it can influence many pro-inflammatory molecules. An inhibitory effect on LDL-induced cytokines and on the expression of adhesion molecules was attributed to α-tocopherol. In fact, this compound also reduced the expression of ICAM-1, VCAM-1 and E-selectin in IL-1β-stimulated endothelial cells [76].

Moreover, a great number of experiments have been carried out to elucidate the effect of triterpenes on atherothrombotic risk factors such as lipid profile, oxidative stress, hyperglycemia, endothelial dysfunction, hypertension and inflammation [54]. In this context, the consumption of triterpene-enriched VOO (particularly in oleanolic acid) improved endothelial function in spontaneous hypertensive rats, by increasing the endothelial NO-mediated relaxation of aortic rings through an enhanced eNOS expression [77]. Moreover, a decrease of COX-2 gene expression was noted in human activated monocytes of subjects submitted to a diet rich in oleanolic acid [78]. Additionally, maslinic acid has shown a potent dose-dependent antioxidant effect on a chemical model of LDL peroxidation, whereas uvaol and erythrodiol acids exhibited both antioxidant and antithrombotic activities [79].

2.3. Ulcerative Colitis (UC) and Crohn´s Disease (CD)

Ulcerative colitis (UC) and Crohn's disease (CD) are the two main types of inflammatory bowel diseases. While UC is characterised by diffuse mucosal inflammation limited to the colon, the CD is characterised by patchy, transmural inflammation, which may affect any part of the gastrointestinal tract [80].

Evidences point that genetic factors, enteric microflora and host response are the causes of inflammation in the two diseases [81]. LPS stimuli of the bacteria lead to the cleavage of IκBα and to the concomitant activation of NF-κB in the epithelial cells (Figure 4). This in turn triggers the inflammatory cell signaling pathways (COX-2 and LOX expression), the release of chemokines and pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and IL-8, thus promoting the recruitment of other leucocytes [82]. At this point, the characteristics of tissue damage will define one of two inflammation pathways: an excessive T helper 1 (T_H1, as stated before, are immunity effectors against intracellular bacteria and protozoa) response, which is associated with CD or alternatively, an excessive T_H2 phenotype that is linked to the development of UC. Secretion of cytokines influences T lymphocytes maturation. In particular, the overproduction of IL-12 shifts the immune response in a T_H1 direction, and the concomitant increased production of IFN-γ, TNF-α, IL-1β, IL-2, and IL-6, resulting in a self-sustaining cycle of activation. In turn, an excessive T_H2 cell response is associated with increased secretion of IL-4, IL-5, IL-10 and IL-13.

It is also believed that a deficient production of transforming growth factor (TGF)-β, IL-10 and other immune-inhibitory cytokines by T_H3 and T regulatory 1 cells (T_R1) precipitates the loss of tolerance of the mucosa, turning it sensitive to ordinary antigens of the microflora [82, 83].

Some studies have shown that a dietary rich in VOO can attenuate bowel inflammation. Hegazi et al. [84] demonstrated that the introduction of VOO in the diet of IL-10 knock-out mices caused modulation of chronic colitis, inhibiting the expression of COX-2 and decreased dysplasia, an early stage of pre-cancer lesion. In dextran sodium sulphate (DSS)-induced colitis rats, the intake of VOO resulted in a reduction of ROS and NO levels, due to the inhibition of iNOS. Moreover, in the same model, rats fed with (n-3) PUFA-enriched VOO exhibited decreased expression of iNOS and of LPS-mediated TNF-α, together with the inhibition of the NF-κB signaling, which is a fundamental piece in the gene activation of the previous mediators [85].

Phenolic compounds have been closely associated to impairment of inflammatory processes in bowel diseases. Recent studies demonstrated the ability of an VOO extract enriched in polyphenols (e.g. oleuropein aglycone, hydroxytyrosol, tyrosol, etc.) to significantly reduce the expression of TNF-α and COX-2, iNOS and a chemokine that promotes the migration and infiltration of monocytes (the chemotactic protein (MCP)-1), in DSS colitis-induced mice. The authors also referred that during the same treatment, the activation of MAPKs (particularly p38 and JNK MAPKs) and the degradation of IκBα were both diminished, while the activity of the peroxisome proliferator activated receptor γ (PPARγ, a key transcription factor in maintenance of gut homeostasis) was increased [86, 87].

The previous results are in agreement with earlier studies that attested a significant improvement on the production of IL-10 and an inhibitory effect on iNOS and COX-2 expression, and p38 MAPK activation in colitis-induced mice fed with hydroxytyrosol-enriched VOO, thus suggesting that hydroxytyrosol is one of the VOO compounds responsible for modulating these proteins [1]. Giner et al. [88] recently proved that oleuropein interacts in the inflammatory pathways associated to bowel diseases. Their study demonstrated that the production of the pro-inflammatory mediators IL-6 and IL-1β was significantly decreased in oleuropein-treated DSS chronic colitis-induced mice, while the IL-10 levels were increased [88].
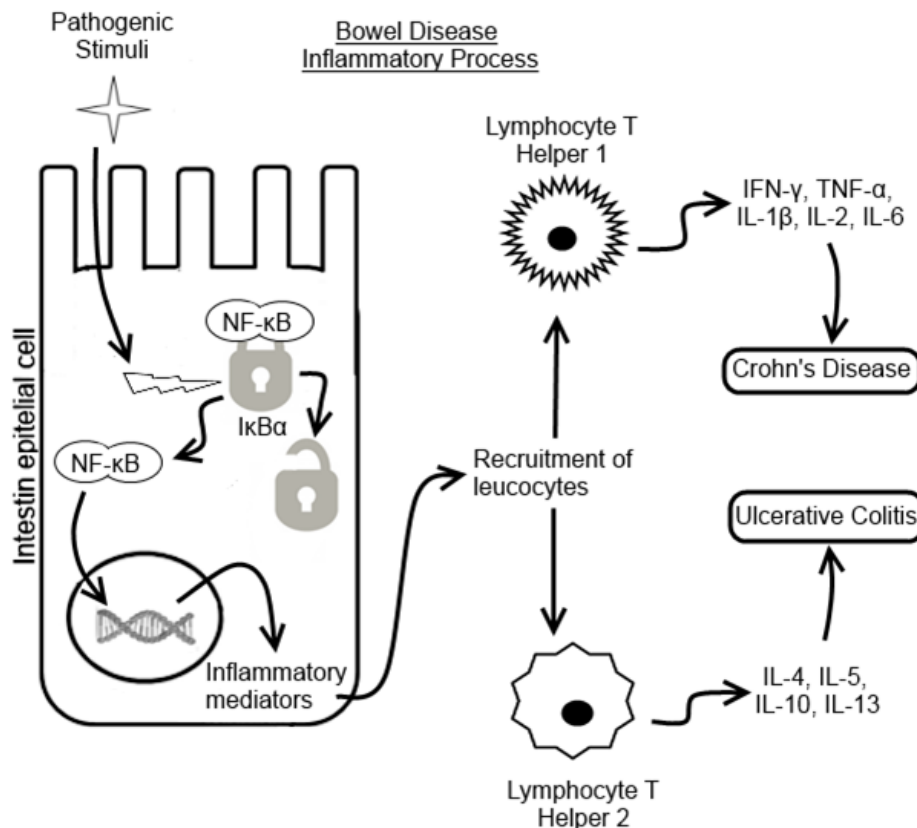

Figure 4. Developing of bowel inflammation. The pathogenic stimulus promotes the degradation of the IκBα, thus releasing the NF-κB. When activated, this nuclear transcription factor migrates to the nucleus where it is responsible for the transcription of several inflammatory mediators genes.

Depending on the mediators produced, one of two pathways can be triggered: 1- activation of the lymphocytes T helper 1 resulting on a Crohn's Disease; 2- activation of the lymphocytes T helper 2 leading to an Ulcerative Colitis.

Additionally, the treatment of chronic colitis mices with oleuropein lowered the expressions of COX-2 and iNOS, the decrease phosphorylation of p38 sub-unity of MAPK pathway and the reduction of NF-κB activation. It was also concluded that oleuropein remarkably stimulated the production of Annexin A1, a potent endogenous anti-inflammatory agent capable of inhibiting COX-2 and iNOS, in injured colon tissue [88].

A recent study demonstrated, for the first time, the protective effects of the VOO unsaponifiable fraction on bowel diseases. Sánchez-Fidalgo et al. [89] demonstrated that unsaponifiable enriched diets were able to block the activation of TNF-α and MCP-1 and also mediate COX-2 and iNOS downregulation, thus ameliorating the first stage of ulcerative colitis. In addition, the authors referred a blockage of the MAPKs and of NF-κB pathways, through decrement of the p38 activation and IκBα degradation, respectively. The consequent reduction of the expression of other mediators (e.g. COX-2, TNF-α, iNOS) was equally shown.

Besides that study, other authors have reported the anti-inflammatory properties of some minor components of the VOO´s unsaponifiable fraction in colitis models. For instance, α-tocopherol effects were assessed in ulcerative colitis mice models demonstrating suppression of serum IL-6 and serum kinase C, as well as promotion of serum IL-10, overall resulting in a decreased colonic damage [90]. Also, β-sitosterol has been demonstrated to inhibit the expression of TNF-α, IL-1β IL-6, COX-2, as well as the activation of NF-κB, in 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitic mice colons [45].

2.4. Other Effects

Cancer

Chronic inflammation also plays an important role in the development and progression of phenomena related with cancer and was estimated to contribute to 15–20% of all cancers [91, 92]. The relevance of inflammation in carcinogenesis was firstly proposed in the 19th century by Rudolf Virchow after verifying the presence of leukocytes within tumors [93]. Presently, it is generally accepted that inflammation is involved at distinct stages of carcinogenesis, particularly in tumor initiation (mutations, genomic instability, and epigenetic modifications) and along progression, by activating tissue repair responses, promoting cells surveillance and proliferation of premalignant cells, by enhancing their survival and causing localized immunosuppression and accumulation of additional mutations, in between others [94]. Moreover, the microenvironment of tumor is characterized by the presence of several inflammatory cells such as neutrophils, eosinophils, lymphocytes, mast cells, dendritic cells, tumor-associated macrophages (TAM) and NKcells. These cells produce PLA2, COX-2, 5-LOX, MMPs and serine and cysteine proteases, as well as membrane perforating agents, reactive oxygen and nitrogen species and also several cytokines (TNF-α, IL-1, IL-6, IL-8, interferons, chemokines) that are associated to DNA damage, increase of mutation rates, development of tumor growth and promotion of metastasis genomic instability. The activation of transcription factors such as NF-κB, nuclear factor erythroid 2 related factor 2

(Nrf2, which regulates environmental stress response by activating the expression of genes for antioxidants and detoxification enzymes) or signal transducers and activators of transcription-3 (STAT3, a protein of the STAT family that mediates the expression of a variety of genes in response to cell stimuli, and thus plays a key role in many cellular processes such as cell growth and apoptosis) are involved in these effects [95].

Epidemiological studies using VOOs have evidenced their preventive effect on some types of cancer, including the breast cancer [96-98]. In particular, VOO was able to improve the efficacy treatment of letrozole and anastrozole, two commercially available therapies for early and late stages of breast cancer, as shown in the human breast cancer MCF-7 cells model. In the same model, a similar treatment promoted the mitochondrial release of cytochrome c and apoptosis, which were mediated by a depletion of reduced glutathione levels, disruption of membrane potential and the increase of lipid peroxidation [99].

The antitumor activity of VOO was also observed in SKBr3, MCF-7 and JIMT-1 breast cancer cell lines, with IC_{50} values ranging from 200 to 300 μg/mL, as measured by MTT assay [100]. Moreover, VOO has a chemopreventive effect in the ulcerative colitis-associated colorectal cancer, as demostrated in a C57BL/6 mice model. Animals feed with VOO presented a minor number of dysplastic macroscopic lesions, a reduction of TNF-α, IL-6 and IFN-γ and COX-2 levels and a decreased expression of iNOS in the colonic tissue [101].

Amongst VOO´s polyphenols, hydroxytyrosol and oleuropein have been recognized as being anti-inflammatory and also modulators of several signal transduction pathways associated with cell survival, proliferation and apoptosis. The mechanisms of hydroxytyrosol include the decreased expression of 5-LOX, reduced synthesis of prostaglandin E2 and alteration of tumour eicosanoid biosynthesis. Apoptosis promotion and prevention of oxidative DNA damage are other described mechanisms for hydroxytyrosol and oleuropein, as concluded from studies performed in human tumour-cell lines, blood mononuclear cells as HL60 cells (the latter a human leukemia cells) [102-104]. Moreover, in cultured endothelial cells, hydroxytyrosol and oleuropein have promoted a suppression of the inflammatory angiogenesis through MMP-9 downregulation, together with an inhibitory effect on COX-2 expression and on the ROS levels [105].

At last, the anticancer effects demonstrated for an unsaponifiable fraction of VOO (in HT-29 human colon adenocarcinoma cells) have been associated to its ability in downregulating COX-2 through PPARγ and NF-kB signaling pathways, apoptosis promotion and modulation levels of p53 suppressor protein (a nuclear protein that functions as a regulator of transcription) [106].

Neurodegenerative Diseases

Mediterranean-style diet has also been related to decreased risks for neurodegenerative diseases [107, 108] and in particular, the dietary component VOO, included in Mediterranean diet, has been reported to promote beneficial neuroprotective effects in Alzheimer's and Parkinson's diseases, stroke, traumatic brain injury and multiple sclerosis [102, 109]. Note also that the protective role of Mediterranean diet against cognitive decline has been related to the attenuation of inflammation [110]. A described mechanism includes the decrease of C-reactive protein levels, which is an inflammatory marker upregulated in Alzheimer's disease associated to the presence of neuritic plaques and neurofibrillary tangles [111].

Despite the protective effects of VOO in neurodegenerative-inflammatory related processes are still scarcely studied, *in vivo* experiments in the mouse model SAMP8 showed

that VOO significantly improved learning and memory [110]. Moreover, oleocanthal, which as stated before exerts anti-inflammatory properties through COX-1 and COX-2 inhibition [112], is also an inhibitor of Aβ-amyloid oligomers and of fibrillization of tau-protein, counteracting functional damage in synapses and the formation of neurofibrillary tangles, respectively [113-116]. Besides that, oleuropein is known to form a non-covalent complex with Aβ-amyloid peptide, thus decreasing or preventing the Aβ-aggregation, and consequently, it counteracts the deposition of these neurotoxic structures [117].

CONCLUSION

VOO has become recognized as an important ingredient of Mediterranean diet due to its claimed health benefits. This chapter summarizes existing information on the beneficial properties of VOO, or of its components, in inflammatory-related diseases such as atherosclerosis, bowel diseases, cancer and neurodegenerative disorders.

The anti-inflammatory activities of VOO includes the inhibition of NF-κB activation, together with the expression decrement of cytokines and chemokines, LOX and COX-2 enzymes, with concomitant lowering levels of prostaglandins, leukotrienes and thromboxanes. Other mechanism includes the decrease of intracellular oxidative stress. Overall, the main VOO´s components that have been associated to its anti-inflammatory effects enclose the oleic acid, phenolic compounds (particularly hydroxytyrosol, oleuropein and oleocanthal) and some unsaponifiable components, namely the α-tocopherol and β-sitosterol.

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