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Phenolic Compounds of Olive by-products and Their Function in the Development of Cancer Cells

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Abstract

Cancer is responsible for the most death-related disease in the world. Epidemiological studies have shown that a lower incidence of certain cancers such as breast, prostate, pancreas and endometrial cancers in Mediterranean countries. The health-conferring benefits of the Mediterranean diet are mainly due to a high consumption of fibre, fish, fruits and vegetables. Olives and olive oil are the main components of the Mediterranean diet. These products contain an abundance of phenolic antioxidants including simple phenols (hydroxytyrosol, tyrosol), aldehydic secoiridoids, flavonoids and lignans (acetoxypinoresinol, pinoresinol). Since antioxidant properties of dietary phytochemicals have well-established roles in disease such as cancer, the present review focuses on recent researches analysing the relationship of olive derivatives in respect to their anticancer and antioxidant mechanism.

Phenolic compounds in olive by-products may initiate biological reactions to affect cancer cells. Studies have shown that olive and olive oil compounds exhibit anti-cancer activity in cancer cell lines, including breast, colon and leukaemia. Their mode of action is described to suppress proliferation by interfering with ERK1/2 activation and reduction of Cyclin D1 expression.

In conclusion, phenolic compounds of olive products may exert a protective activity and might have a therapeutic potential for the treatment of cancer.

Key words: Olive, olive oil, phenolic compounds, antioxidant capacity, anticancer activity

INTRODUCTION

Olive (*Olea europaea*) is one of the most widespread and economic important woody plants in the Mediterranean and Middle Eastern regions. Olive and its products are principle components of the Mediterranean diet, which provide numerous health benefits [1-4]. The health-promoting properties of the Mediterranean diet have been largely attributed to the antioxidant and free radical-scavenging activity of polyphenols contained in the dietary components, especially in olive fruit and oil [4]. Due to these biological actives, there is an increasing interest in relationship between phenols found in olive products and prevention of cardiovascular disease and cancer [5]. The regular consumption of olive products was suggested to be one of the factors associated with a lower incidence of cancers including breast, prostate, pancreas and endometrial in the Mediterranean countries [6]. It was reported that evidence for the protective role of olives was also derived by comparing olive oil consumption in the Mediterranean countries to that of the United States. Mediterranean populations consume 20 times more olive oil than Americans; correspondingly, their cancer risk is at least half [7]. In another study, more direct evidence of anticancer activity of olive oil has been published by [8]. Since antioxidant properties of dietary phytochemicals have well-established roles in disease such as cancer, the present review focuses on recent researches analysing the relationship of olive derivatives in respect to their anticancer and antioxidant mechanism.

Chemistry of phenols in olive and olive oil

The major phenolic compounds identified and quantified in olive and olive oil belong to three different classes: simple phenols (hydroxytyrosol, tyrosol); secoiridoids (oleuropein, the aglycone of ligstroside and their respective decarboxylated dialdehyde derivatives) and the lignans [(+)-1-acetoxypinoresinol & (+)- pinoresinol]. All three classes have potent antioxidant properties [5,9,10].

The types of phenols in olive fruit are different from those of the olive oil. Also, the phenolic content of the olive fruit changes as it grows and develops. The olives mainly contain the polar glycosides oleuropein and ligstroside. When the fruits are about 6 months old, the major phenolic components are secoiridoid glucosides, called ligstroside and oleuropein glucoside, but these are not detected in the olive fruit harvested at maturity. As the olive fruit matures the concentration of oleuropein decreases and hydroxytyrosol increases [11].The glucoside precursors are present in the pericarp, but as the olive reaches maturity they are deglycosylated by glucosidase enzymes, releasing the free secoiridoids [12,13].

Oleuropein is the major phenolic compound in olive fruit, which is responsible for the bitterness of fresh green olives. Oleuropein is the ester of hydroxytyrosol with the oleoside 11-methylester characterized by a hexocyclic 8,9-olefinic group [14]. Oleuropein- and ligstroside- aglycones are formed by removal of the glucose moiety from the oleuropein- and

ligstroside-glycoside by bglucosidase during ripening. Those aglycones and their various derivatives are the most abundant phenols in olive oil [11,13,14].

The polar compounds hydroxytyrosol and tyrosol are the end products of hydrolysis of oleuropein- and ligstroside-aglycones or their derivatives in olives and olive oil [13,14]. Hydroxytyrosol and tyrosol are structurally identical except that hydroxytyrosol posses an extra hydroxy group in the *meta* position. Hydroxytyrosol is the major phenolic component in olive oil [11].Verbascoside is a slightly more complex molecule synthesized by grouping caffeic acid with rutinose and hydroxytyrosol [15]. Verbascoside is a disaccharide, comprising glucose and rhamnose, bound to a hydroxytyrosol and hydroxycinnamic acid molecule, respectively [14]. Basic phenolic compounds in olive and its products are shown in Table 1 and Figure 1.

Table 1. Predominant polyphenol composition in olive fruit and olive oi

Polyphenol	Olive fruit mg/g [a]	Olive oil mg/kg [b]
Oleuropein	2.4	7.7*
Hydroxytyrosol	0.5	1.3
Tyrosol	0.1	2.1
Verbascoside	3.2	ND
Demethyloleuropein	0.14	ND
Apigenin-7-glucoside	0.40	ND
Luteolin-7-glucoside	0.13	ND
Oleoside-11-methylester	0.5	4.0

Compiled from: [a]Romani et al [32]; [b]Gómez-Alonso et al [33]
ND = Not detected; *value for oleuropein aglycone

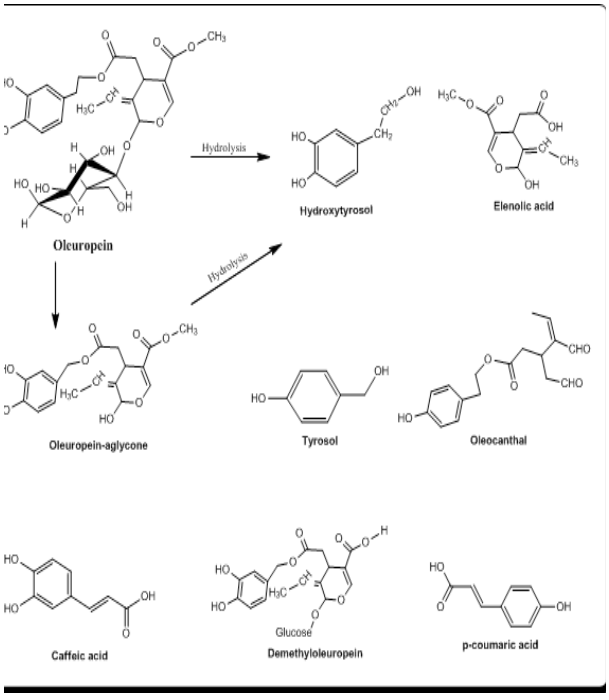

Figure 1. Chemical structures of predominant olive plant polyphenols.

Antioxidant Effect

The interest in the antioxidant properties of phenolic components present in olives and olive oil was investigated in a range of studies [4,11,16].Phenols are compounds with an aromatic ring structure with one or more hydroxyl groups. Phenols with two or more hydroxyl groups show antioxidant capacity in vitro, whereas phenols with one hydroxyl group have little or none [17]. In particular, the ortho-diOH substitution confers a high antioxidant capacity, whereas single hydroxyl substitutions, as in the case of tyrosol, provide no activity [18].

Black olive pericarp extract has a higher concentration of phenolic compounds and a higher antioxidant capacity than green olive pericarp extract. It has been known for many years that compounds with a catechol group exhibit antioxidant activity. Hydroxytyrosol and oleuropein show dose-dependent activity and are considered potent antioxidants, demonstrating activity in the micro-molar range. Olive oil phenols are capable of scavenging free radicals produced in the fecal matrix, which is thought to explain the epidemiological data suggesting a colonic chemoprotective effect of olive oil [13].

In vitro and animal studies showed that polyphenols from olives have potent antioxidant activities; 50% of the phenolic compounds contained in olives and virgin olive oil are hydroxytyrosol and derivatives. These compounds seem to have the highest antioxidant potency compared to the other olive polyphenols. The radical scavenging potency of o-methylated hydroxytyrosol was similar and that of the 3-o-glucuronide conjugate was more potent than hydroxytyrosol in vitro, whereas the monosulphate conjugate of hydroxytyrosol was almost devoid of its radical scavenging activity [17]. Review of the human intervention studies showed that olive polyphenols (e.g. hydroxytyrosol and oleuropein) decreased the levels of oxidized-LDL in plasma and positively affected several biomarkers of oxidative damage [13,19,20].

In-vitro and *ex-vivo* models demonstrated that olive oil phenolics have antioxidant properties higher than that of vitamin E on lipids and DNA oxidation. Also, olive oil phenolic compounds inhibited platelet-induced aggregation and it was reported to enhance the mRNA transcription of the antioxidant enzyme glutathione peroxidase [21]. The identification of lignans as major antioxidant components of the phenolic fraction of olive oil is also of considerable interest. Owen et al [12] stated that lignans in animal cellular and metabolic studies possess important biological effects, which may contribute to their potential as chemopreventive agents.

Bioavailability

Vissers et al [17] investigated the bioavailability of olive and olive oil phenolics in animal human studies resulting in an increase of antioxidant activity. Absorption is confirmed by the recovery of tyrosol and hydroxytyrosol in urine after intake of olive and olive oil phenols.

On the basis of the scavenger capacity of phenolic compounds on free radicals generated by the faecal matrix and those induced in the intestinal epithelium cells. Tyrosol and hydroxytyrosol and their derivatives are absorbed by humans in a dose-dependent manner with the phenolic content of the olive oil administered. Concerning the dose-response relationship, urinary concentrations of tyrosol were dependent on the administered tyrosol dose, whereas hydroxytyrosol urinary concentrations tended to accumulate. In fact, some preliminary reports support the view that the 3-o-glucuronide of hydroxytyrosol shows stronger activity as a radical scavenger

than hydroxytyrosol itself [21]. The protective effects of hydroxytyrosol and oleuropein were demonstrated through the assessment of various markers of LDL oxidation, including a reduced formation of short-chain aldehydes and of lipid peroxides, by a higher vitamin E content in the residual LDL, and by a reduced formation of malondialdehyde-lysine and 4-hydroxynonenal-lysine adducts, indicating protection of the apoprotein layer [18].

Vissers et al [18] reported that apparent in vivo absorption of the ingested olive oil phenols was more than 55–66 mol% in humans. Tuck et al [22] showed that bioavailability of radiolabelled tyrosol and hydroxytyrosol was 71–99% compared to intravenously administered tyrosol and hydroxytyrosol in rats.

Anticancer Activity

Since recent epidemiological evidence and animal studies suggest olive and its byproducts show anticancer activity, the researchers have focused on possible mechanisms to explain this phenomenon. Oxidation of proteins, DNA, and lipids contribute to cancer development, and consumption of antioxidants is believed to reduce the risk of mutagenesis and carcinogenesis [23]. Antioxidants are present in olive and olive oil, fruits, and vegetables that constitute a large part of the Mediterranean diet [13].

Olive oil intake has been shown to induce significant levels of apoptosis in various cancer cells including breast, prostate and colon. These anti-cancer properties are thought to be mediated by phenolic compounds present in olive [13,24]. A polyphenolic fraction extracted from olive oil was investigated on proliferation, the cell cycle distribution profile, apoptosis, and differentiation of the promyelocytic leukemia cells (HL60) [25]. A phenolic extract showed antiproliferative effect in a time- and concentration-dependent manner in HL60 cells. Cell growth was blocked at a concentration of 13.5 mg/l and the treatment with it induced apoptosis and differentiation. Determination of the cell cycle distribution revealed an accumulation of cells in the G(0)/G(1) phase. These results support the hypothesis that polyphenols play a critical role in the anticancer mechanism of olive oil.

These beneficial health effects of olive and olive oil attributed, at least in part, to the presence of hydroxytyrosol and oleuropein [24]. Most recent research has focused on anticancer activity of major phenolic compounds in olives and its byproducts. Fabiani et al [26]. has reported that hydroxytyrosol is capable of protecting cells from hydrogen peroxide damage and DNA from peroxynitrite-induced damage, blocking cell cycle progression at the G phase, and inducing apoptosis in the leukaemia (HL60) and colon adenocarcinoma cells (HT29). At concentrations ranging from 50 to 100 mmol/l, hydroxytyrosol induces an appreciable apoptosis in HL60 cells after 24 h of incubation and that concentrations arrest the cells in the G(0)/G(1) phase with a concomitant decreasing in the cell percentage in the S and G(2)/M phases.

In another study, the differentiation inducing ability of hydroxytyrosol was found on HL60 with a maximum effect (22% of cells) at 100 mmol/L after exposure for 72 h. Among the proteins involved in the regulation of the cell cycle, hydroxytyrosol reduced the level of cyclin-dependent kinase (CDK) 6 and increased that of cyclin D3. Whereas the expression of p21WAF1/Cip1 and p27Kip1 was increased at both protein and mRNA, p15 was not altered by hydroxytyrosol [27].

Antiproliferative effect of hydroxytyrosol was investigated on human colon adenocarcinoma by Corona et al [28].They found that this compound inhibited proliferation by inducing a cell cycle block in G2/M. These antiproliferative effects were preceded by a strong inhibition of extracellular signal-regulated kinase (ERK) 1/2 phosphorylation and a downstream reduction of cyclin D1 expression, rather than by inhibition of p38 activity and cyclooxygenase-2 (COX-2) expression.

In-vivo and *in-vitro* studies on the activity of oleuropein have revealed a range of biologically attributes in addition to its antioxidant properties, such as antiangiogenic action, inhibition of cell growth, motility and invasiveness. Oleuropein was found to cause cell rounding, which disrupts the cell actin cytoskeleton. Moreover, oleuropein affects and disrupts purified actin filaments, providing direct antitumor effects due to cell disruption [29].

In breast cancer field, Menendez et al [30] investigated the possible effects of phenolic fractions from extra-virgin olive oil (EVOO) and its main constituents on SKBR3 cell line. EVOO polyphenols lignans, flavonoids and secoiridoids was found to drastically suppress fatty acid synthase (FASN) protein expression in HER2 gene-amplified SKBR3 breast cancer cells. Similar results were observed in MCF-7/HER2 cells, a well-characterized up-regulator of FASN expression in aggressive sub-types of cancer cells. On the other hand, single phenols and phenolic acids of EVOO acids failed to modulate FASN expression in SKBR3 and MCF-7/HER2 cells. It has been suggested that phenolic fractions may induce anti-cancer effects by suppressing the expression of the lipogenic enzyme FASN in HER2-overexpressing breast carcinoma cells.

It was reported that oleuropein or hydroxytyrosol inhibit cell proliferation and induce cell apoptosis in MCF-7 breast cancer cells. These compounds exhibit statistically significant block of G1 to S phase transition manifested by the increase of cell number in G0/G1 phase [24].

The inhibition effect of hydroxytyrosol and oleuropein on E2-induced molecular mechanisms in breast cancer cell (MCF-7) was tested by Sirianni et al [31].According to luciferase gene reporter experiment results, these compounds were not involved in estrogen receptor alpha (ERalpha)-mediated regulation of gene expression. However, further experiments pointed out that both oleuropein and hydroxytyrosol showed inhibition effect on E2-dependent activation of extracellular regulated kinase1/2 belonging to the mitogen activating protein kinase (MAPK) family.

CONCLUSION

A high percentage of olive oil phenols absorbed after ingestion are believed to act in the blood vessels to prevent LDL oxidation and in tissues to protect against DNA damage. Since the antioxidant activity of hydroxytyrosol and oleuropein is dose dependent, the amount of olive oil consumed is likely to affect its chemoprotective and anticancer effects.

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