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Research Article

Hydroxytyrosol: A natural compound with promising pharmacological activities

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ABSTRACT

Hydroxytyrosol is a phenolic phytochemical with antioxidant properties *in vitro*. It is a natural compound that can be found in olive leaves and oil. The main dietary source of hydroxytyrosol is extra virgin olive oil. Due to its bioavailability, chemical properties and easy formulation along with its lack of toxicity, hydroxytyrosol is considered an excellent food supplement by the nutraceutical and food industries. The purpose of this review is to discuss the potential therapeutic effects of hydroxytyrosol *in vivo*. To do so, we conducted an electronic search in PubMed and other literature databases using "hydroxytyrosol", "beneficial effect/s", "pharmacology" as keywords. From this search, we found that hydroxytyrosol has anti-inflammatory, anti-tumor, antiviral, antibacterial and antifungal properties. Hydroxytyrosol also improves endothelial dysfunction, decreases oxidative stress, and is neuro- and cardio-protective. Due to all these biological properties, hydroxytyrosol is currently the most actively investigated natural phenol. The evidence presented in this review suggests that hydroxytyrosol has great pharmacological potential.

1. Introduction

Significant beneficial effects of extra virgin olive oil are attributed to its phenolic constituents and fatty acid composition (Auñon-Calles et al., 2013a). One phenolic compound is hydroxytyrosol (IUPAC name: 4-(2-Hydroxyethyl)-1,2-benzenediol), a molecule with antioxidant activity (Stefanon and Colitti, 2016). Hydroxytyrosol (HT) can be extracted from olive leaves and oil, is stable in the free form and penetrates readily into tissues (Bonetti et al., 2016). The chemical formula is $C_8H_{10}O_3$ and is identical to tyrosol except for an extra hydroxyl group in meta-position in the aromatic ring (Fig. 1). HT is derived from hydrolysis of oleuropein (Fig. 1) during maturation of olives (Santos et al., 2012). It is soluble in lipids, but is also slightly soluble in water. It can exist as a simple phenol, or as acetate or secoiridoid derivatives (Bonetti et al., 2016; Hu et al., 2014).

HT has anti-inflammatory, anti-atherogenic and anti-thrombotic properties. *In vitro* studies show that, besides its antioxidant activity, HT can improve endothelial dysfunction, lipid profiles and hemostatic profiles, and has anti-inflammatory properties (Stefanon and Colitti, 2016). It may therefore be considered a neuroprotective (Schaffer et al., 2007), cardioprotective (Visioli, 2012) and chemopreventive agent (Visioli et al., 2004). HT may interact with proteins involved in control of the cell cycle and in gene expression and could therefore have anticancer properties (Serreli and Deiana, 2018). Here we discuss the

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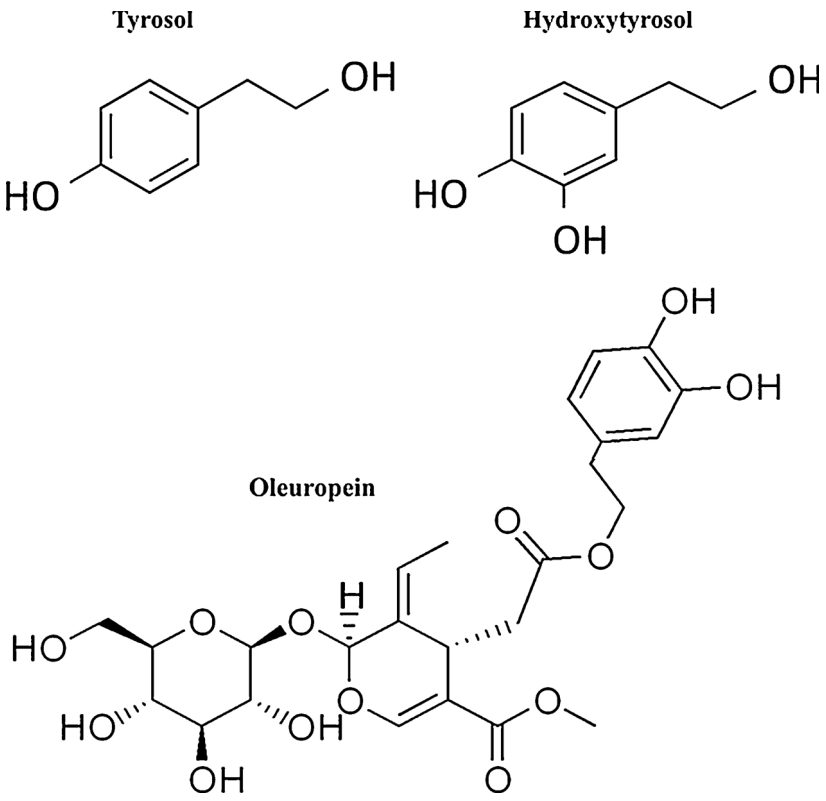

Fig. 1. Schematic representation of the chemical structure of tyrosol, hydroxytyrosol and oleuropein.

potential therapeutic effects of HT and its pharmacokinetics.

2. Methods

This is a "qualitative review" of the effects of HT on human health. All the information used for the preparation of this document is based on data from original documents and reviews. We selected the most relevant studies on the pharmacological aspects of HT. We conducted an electronic search in PubMed database, using the following string: hydroxytyrosol [text word] AND ("beneficial effects" [text word] OR "beneficial effect" [text word] OR "pharmacology" [text word]).

Only articles in English containing detailed characterization of HT and its effects on *in vitro* and *in vivo* models, published until November 2019 were included. The references lists of all articles were scanned to retrieve additional relevant research. From this search we identified 481 records, of them, 381 deal with HT activity and discussed in the review only the most relevant papers as personally evaluated by the authors.

3. Results and discussion

3.1. Pharmacokinetics, pharmacodynamics and safety profile

Extra virgin olive oil, a major component of the so-called Mediterranean diet, has various health benefits, mainly due to the phenolic compound hydroxytyrosol (HT) (Rodríguez-Morató et al., 2016). Bioavailability studies have established that after ingestion, the absorption of HT from olive oil is dose-dependent and has significant biological effects (Serreli and Deiana, 2018).

A study by Corona et al. (2006) established that transformation of HT, tyrosol and their forms conjugated by hydrolysis in gastric conditions occurs in the small intestine and results in increased serum levels of free HT and tyrosol. Similarly, oleuropein is rapidly degraded to HT by colon microflora (Corona et al., 2006; Hu et al., 2014).

Analysis by HPLC revealed that HT is converted by enzymes into oxidized and/or methylated derivatives, *i.e.* an O-methylated derivative of HT, glucuronides of HT and a newly discovered glutathionylated conjugate of HT (de Bock et al., 2013).

HT potentially may have effect on health status. For instance, in a *in vivo* study it was demonstrated that the administration of HT to rabbits fed with high fat diets reduces the atherosclerosis development (González-Santiago et al., 2006) and studies in cellular models demonstrated the ability of HT to arrest the cell cycle and induce apoptosis, an important property for possible anti-cancer applications (Fabiani et al., 2002).

HT has also an excellent safety profile, with no adverse effects in a mouse model, even at a very high doses (Christian et al., 2004). Toxicological evaluation and *in vitro* genotoxicity studies have classified HT as a non-mutagenic, non-genotoxic compound with NOAEL (No Observed Adverse Effects Level) classification, suitable for long-term consumption (Auñon-Calles et al., 2013a, 2013b).

Due to all these significant biological properties, HT is currently the most actively investigated natural phenol. The safety profile of HT makes it an excellent food supplement for the nutraceutical and food industries (Hu et al., 2014).

3.2. Effects on cancer cells

HT has been tested to evaluate its activity in cancer cells in several works.

In MCF-7 (Michigan Cancer Foundation-7) breast cancer cell model, HT decreases the proliferation (Chimento et al., 2014). This effect may be linked to its pro-apoptotic activity (Han et al., 2009). The ability of HT to prevent the proliferation of MCF-7 cells does not include a typical ERα-mediated gene regulation mechanism but the ERK1/2 inhibition (Sirianni et al., 2010). This is also demonstrated by the fact that HT induces apoptosis in ER-negative SK-BR-3 breast cancer cells by antagonizing the G protein-coupled estrogen receptor (GPER) that then leads to apoptosis (Chimento et al., 2014).

HT has also been tested on other cancer cells type like colon,

prostate or thyroid cancer cells. Interestingly, although HT has anti-oxidant effects in physiological conditions, in colon cancer cells it induces apoptosis through the generation of H_2O_2 and reactive oxygen species (ROS). This unexpected behavior might be linked with the dysregulated expression of the catalase (that convert H_2O_2 into water and oxygen) in tumor cells (Luo et al., 2013; Sun et al., 2014; Toteda et al., 2017). Other mechanisms have been proposed to be involved in anti-proliferative effect of HT. In fact, it was demonstrated that HT leads to the inhibition of the Akt, NFκB, STAT3, and EGFR signaling pathways in several cancer cell lines: pancreatic, prostate or thyroid (Terzuoli et al., 2016; Zhao et al., 2014; Zubair et al., 2017).

3.3. Anti-inflammatory effect

Inflammation is a protective response to damaged cells, irritants or pathogens, involving immune cells and molecular mediators. All current anti-inflammatory agents (statins, corticosteroids, nonsteroidal anti-inflammatory drugs and disease-modifying anti-rheumatic drugs) not only have toxic risk but also fail to deal with the underlying inflammatory state (Kapoor et al., 2014). HT may act as a strong anti-inflammatory agent (Borrie and Kim, 2017), inhibiting lipopolysaccharide-mediated expression of inflammatory cytokines *i.e.* TNF-α and IL-1β, thus suppressing the inducible nitric oxide synthase/nitric oxide and the cyclooxygenase/prostaglandin E2 pathways (Martínez et al., 2019).

Further studies have established that at nutritionally appropriate concentrations, HT shows anti-inflammatory properties by suppressing the activity and expression of MMP-9 and COX-2 enzymes in activated human monocytes (Scoditti et al., 2014). It also enhances anti-inflammatory effects by decreasing the levels of pro-inflammatory cytokines IL-6 and TNF-α, as observed in animal models (Ramírez-Expósito and Martínez-Martos, 2018).

3.4. Anti-oxidant effect

Olives are a natural source of antioxidants that prevent oxidative stress caused during development of diseases like cancer, coronary heart disease or neurodegenerative diseases. HT has the strongest antioxidant activity among the various olive phenols due to the electron donating capacity of its hydroxyl (OH) groups in ortho position, along with its capacity to form stable hydrogen bonds with phenoxyl radicals (De La Torre-Carbot et al., 2005; Fitó et al., 2005). The protective effect of HT against oxidation of low-density lipoprotein (LDL) is even evident at very low concentrations *in vitro* (Rietjens et al., 2007). The anti-oxidant effect of HT does not depend only on the capacity of scavenging oxidant chemical species, but it also depends on the ability to stimulate the activity and synthesis of anti-oxidant enzymes. In fact, antioxidants, *in vivo*, can either generate reactive species (oxidant) and/or be electrophilic (anti-oxidant). The pro-oxidant beneficial effects depends on the concentrations reached *in vivo*, that are sufficient to cause the activation of signaling pathways that recognize the presence of free radicals. Therefore, the low concentrations of antioxidants in food and beverages might be positive (Forman et al., 2014). In particular, HT induces the synthesis and translocation to the nucleus of Nrf2, then promoting the transcription of several genes that encode anti-oxidant response elements such us DNA-repair proteins or phase II detoxifying enzymes (Zrelli et al., 2011).

3.5. Neuroprotective effect

The neurochemical properties of HT suggest that it may enhance the neuro-protective efficiency of monoamine oxidase (MAO) inhibitor treatment in patients with Parkinson's disease. Since HT is a neutral phenol, when administered it diffuses freely in total body water and central neurons. Consistently, oral administration of HT to laboratory rats causes a dose-related increase in HT and its metabolites in brain tissues (Nicolaïew et al., 1998). When administered by systemic injection, HT can also be identified in striatal microdialysate (Vissers et al., 2001).

Monoamine oxidase inhibition is thought to slow progression of Parkinson's disease by decreasing production of the autotoxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde, however it generates toxic dopamine oxidation products like 5-S-cysteinyl-dopamine. HT is reported to decrease levels of monoamine oxidase inhibition products (Goldstein et al., 2016). These results suggests that HT might have neuroprotective effects.

3.6. Cytoprotective and mitotic effect

Recent studies report that HT may induce cell proliferation and cyto-protection against oxidative injury (Zrelli et al., 2011). Similar experiments have shown that HT and other olive oil phenols, when added to standard cell culture media *i.e.* Roswell Park Memorial Institute (RPMI) medium, Minimum Essential Medium (MEM) or Dulbecco's Modified Eagle Medium (DMEM), instantly induce H_2O_2 release in the micromolar range (Fabiani et al., 2012). At micromolar concentrations, H_2O_2 has the ability to stimulate proliferation of many cell types (Halliwell and Gutteridge, 2015). This is further confirmed by long-term exposure of alveolar epithelial cells in primary culture to a continuous flux of 10 μM/h H_2O_2 that eventually results in enhanced cell proliferation (Sigaud et al., 2005). The same effects on cell proliferation were observed when bovine aortic endothelial cells were exposed to low concentrations of H_2O_2 (Ruiz-Ginés et al., 2000). The effect of HT in inducing H_2O_2 production may explain its effects on cell proliferation, wound healing and cytoprotection. These abilities of HT could play a role in the treatment of diseases that involve cell injury leading to inflammation (Schaffer and Halliwell, 2011).

3.7. Immunomodulatory effect

At non-cytotoxic concentrations, HT inhibits release of hexosaminidase from peritoneal mast cells (a type of granulocyte of the immune and neuroimmune systems, important for allergic response) after stimulation of mast-cell response (Persia et al., 2014). It also inhibits release of histamine from mast cells, and this inhibitory effect is stronger than that of other similar compounds, like oleuropein (Singh et al., 2011; Weng et al., 2012).

HT also reduces allergic response by binding allergenic molecules and making them less recognizable by the immune system (Singh et al., 2011). It has been established that major olive oil phenols like oleuropein and HT prevent degranulation of mast cells by immune and non-immune pathways and may therefore play a role in the treatment of mast-cell-mediated disorders (Persia et al., 2014).

3.8. Anti-viral and anti-microbial effect

In addition to its anti-oxidant and anti-inflammatory properties, HT also shows anti-HIV activity *in vitro*. It inhibits the viral integrase enzyme and fusion of the viral envelope with host cells (Lee-Huang et al., 2007). Although HT is not considered an efficient oral anti-retroviral agent, because of its low intrinsic activity, it is able to repress the replication of numerous HIV-1 strains, as well as drug-resistant isolates and viruses, in additive and synergistic ways with antivirals and without harmful effects *in vivo* (Lee-Huang et al., 2007).

HT has also been considered a potential microbicide (Bedoya et al., 2016). Since the 1970s, there has been much evidence in support of the antimicrobial and bacteriostatic activity of olive extracts (Walter et al., 1973). In particular, more recent studies have shown that HT has a potent antimicrobial activity especially against *Clostridium perfringens*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica*, *Yersinia* sp., and *Shigella sonnei* (Medina et al., 2006).

3.9. Anti-adipogenic effect

Olive oil is currently studied for its potential effects in the treatment of obesity and metabolic disorders (Scoditti et al., 2015). In particular, HT prevents the inhibition of adiponectin expression by TNF-α. Adiponectin is a specific protein secreted by adipocytes and has anti-diabetic, anti-inflammatory and anti-atherogenic properties (Scoditti et al., 2012). HT also inhibits differentiation of primary pre-adipocytes, promotes lipolytic activity and causes apoptosis in differentiating pre-adipocytes. Molecular studies have also shown that HT controls the expression of several adipogenesis-associated genes (Kurylowicz et al., 2015). This anti-adipogenic activity of HT during cell differentiation is related to a delay in cell cycle progression as well as down-regulation of SREBP-1c, a transcription factor necessary for lipogenesis (Kurylowicz et al., 2015; Stefanon and Colitti, 2016). HT therefore plays a protective role against fat accumulation and obesity and prevents diseases caused by these factors.

3.10. Conclusions

In this review, we report evidence of the biological significance of HT. HT may have cardioprotective, antiatherogenic, antiviral, antibacterial, anticarcinogenic, neuroprotective and anti-obesity effects. This review confirms previous indications and reveals new aspects and therapeutic potential. In the future, it is our intention to obtain HT from natural olive extracts in order to conduct *in vitro* and *in vivo* studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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