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Basic nutritional investigation

Effects of olive polyphenols administration on nerve growth factor and brain-derived neurotrophic factor in the mouse brain

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ABSTRACT

Objective: Polyphenols are chemicals derived from plants known to possess antioxidant and anti-inflammatory properties. High intake of fruit and vegetables is believed to be beneficial to human health. Various studies have suggested that dietary polyphenols may protect against cancer and cardiometabolic and neurodegenerative diseases. Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are neurotrophins that play key roles in brain cell development, growth, and survival. The aim of this study was to investigate whether or not administration of olive (*Olea europaea* L.) polyphenols could have an effect on NGF and BDNF content and the expression of their receptors, TrkA and TrkB, respectively, in the mouse brain.
Methods: NGF and BDNF were measured by enzyme-linked immunosorbent assay. TrkA and TrkB were measured by Western blotting.
Results: We found NGF and BDNF elevation in the hippocampus and olfactory bulbs and a decrease in the frontal cortex and striatum. These data were associated with potentiated expression of TrkA and TrkB in the hippocampus and olfactory bulbs but no differences between groups in the striatum and frontal cortex. Polyphenols did not affect some behavioral mouse parameters associated with stressing situations.
Conclusions: Altogether, this study shows that olive polyphenols in the mouse may increase the levels of NGF and BDNF in crucial areas of the limbic system and olfactory bulbs, which play a key role in learning and memory processes and in the proliferation and migration of endogenous progenitor cells present in the rodent brain.

Introduction

Olive oil, an important component of the Mediterranean diet, is known to possess antioxidant effects, probably due to oleic acid and polyphenols such as oleuropein and hydroxytyrosol [1]. Half of the phenolic compounds contained in olive leaves, olives, and virgin olive oil are hydroxytyrosol and its derivatives. Hydroxytyrosol is the major olive polyphenol consumed and well absorbed in humans. It is considered to have the highest antioxidant potency compared with other olive polyphenols. Experimental findings have shown that olive leaf extract attenuates cardiac, hepatic, and metabolic changes in high carbohydrate-, high-fat–fed rats [2]. In humans, the role of dietary olive oil in preventing cardiometabolic diseases has been proposed [3,4]. It also inhibits in vitro platelet aggregation in human whole blood [5]. In humans, olive polyphenols decreased the plasma levels of oxidized-low-density lipoprotein and positively affected several biomarkers of oxidative damage (reviewed in [6]). It also has been shown that ingestion of olive oil may be beneficial in reducing postprandial triglyceride concentrations when associated with physical exercise [7]. An in vitro study showed that methanol extract from olives, rich in phenolic compounds,

Sara De Nicoló, Luigi Tarani, Mauro Ceccanti, Andrea Vania, George N. Chaldakov, and Marco Fiore planned the experimental protocols. Sara De Nicoló, Mariateresa Maldini, Fausta Natella, and Marco Fiore performed the experiments.

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exhibits gastric cancer preventive efficacy by limiting cell proliferation, inducing cell death, and suppressing inflammation in stomach cancer cells [8]. Extra virgin olive oil also may improve learning and memory in a mouse model of Alzheimer's disease [9,10], acting as a neuroprotective agent.

Neuroprotection in mammals is finely regulated by neurotrophic factors [11,12]. Neurotrophic factors are proteins that stimulate the neuronal survival, development, and functions [13,14]. Neurotrophic factors act by preventing the associated neuron from initiating programmed cell death, thus allowing the neurons to survive [13]. Neurotrophic factors also induce differentiation of progenitor cells to form neurons, a process known as neurogenesis [15–17]. Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are the best studied neurotrophic factors [12,18,19]. NGF and BDNF play a crucial role in the survival and development of specific peripheral and brain neurons [14,19]. Both are produced and released by a variety of cells localized in the central and peripheral nervous systems and by cells of the immune and endocrine systems [20,21]. NGF and BDNF also play key roles in the fine-tuning of learning and memory performances and in some behavioral processes associated with stress situations [22–24], including exposure to ethanol [25]. In particular we have shown that prenatal ethanol in the mouse may disrupt brain NGF and BDNF levels; however, these changes were reduced when ethanol was administered as red wine [24–26]. We discussed these findings as an effect of the antioxidant "protective" properties of the polyphenols contained in the red wine.

Thus the aim of the present study was to investigate whether or not administration of polyphenols per se may have an effect on NGF and BDNF in the hippocampus, frontal cortex, striatum, and olfactory bulbs of the brain of CD-1 adult male laboratory mice. We also studied the NGF and BDNF receptors, TrkA and TrkB, respectively, in the same brain areas [27–29]. For this purpose, we administered for 10 d a blend of 10 mg/kg of polyphenols extracted from olive residues (pomace) obtained following olive pressing in the preparation of extra virgin olive oil. Extract obtained from olive pressing residues is particularly rich in polyphenols (see Methods section). We also measured mouse blood reduced glutathione level (GSH) for assessing oxidative stress [30], the levels of hydroxytyrosol in the serum, and some behavioral parameters for investigating possible toxic effects of polyphenol administration on pain sensitivity and anxiety as the hot-plate and the Porsolt forced swimming tests.

Methods

Animals and polyphenols administration

CD-1 outbred male mice were housed singly in Plexiglas cages (33 × 13 × 14 cm) under standardized conditions with pellet food (enriched standard diet purchased from Mucedola, Settimo Milanese, Italy). A 12L:12D lighting regime was used. CD-1 outbred mice were used because this strain shares some aspects with the *Mus musculus* mouse living in nature and because the CD-1 strain is one of the most common mouse strains used for behavioral studies [31,32]. Animals were divided in two groups, polyphenol-administered animals (n = 6) and their respective controls (n = 6). The polyphenol group received intraperitoneal injections (i.p.) following methods previously described [33–35] for 10 consecutive days 10 mg/kg a mix of polyphenols extracted from the olive residues (pomace). Such residues were obtained during the production of extra virgin olive oil and dissolved in saline. Control group received i.p. for 10 consecutive day saline solution. Polyphenols (Phenolea Active Complex® www.phenofarm.it) were purchased from Leadergy Light, Italy. Animals' body weight was measured on days 0, 5, and 10 of the experimental schedule. Food and water consumption were measured daily. All efforts were made to minimize and reduce animal suffering and to limit the number of animals used. All animal experiments were carried out following the procedure described by the guidelines of the European Community Council Directive of 1986 (86/609/EEC) and all experiments were authorized by the local ethical committees (Ministero Salute, Regione Lazio, following the Decreto Legislativo 116/92; no permit number or approval ID are required because we only inform the committees of our experimentations without receiving any reply in case of positive answer).

Polyphenol description

The polyphenols used in the present study, as indicated by the manufacturer, derive from a natural standardized olive pulp (*Olea europaea* L.) extract obtained by an eco-sustainable patented mechanical process. It is a byproduct of the olive residues (pomace) obtained following olive pressing during the production of extra virgin olive oil. Specifications of the mixture, as indicated by the manufacturer, are shown in Table 1. We used a blend of phenolic compounds because it has been proposed that most of the health benefits associated with virgin olive oil are due to its minor components [36]. Indeed, mixtures of biophenols are supposed to possess a stronger action for counteracting different stages of oxidative damage instead of single compounds, due to their possible synergisms in scavenging hydroxyl radicals than the well-known and studied hydroxytyrosol [37].

Serum hydroxytyrosol measurement

Hydroxytyrosol was measured following methods previously described with minor modifications [38]. The serum for hydroxytyrosol measurement was immediately prepared by centrifugation at 1500g for 30 min at 20°C. Serum aliquots were acidified at pH 3.0 adding formic acid and stored at –80°C. Before analysis, the aliquots of serum from each animal were thawed and pooled to have 0.8 mL samples. The samples were added, with salicylic acid (400 ng) as internal standard and deproteinized by the addition of 3 mL of ice-cold acetonitrile. After vortexing, the samples were centrifuged at 14 000g for 15 min at 4°C. The supernatants were dried using a rotary evaporator under vacuum at 40°C. The dried samples were dissolved in 0.4 mL of MeOH 50% and extracted by SPE using Oasis HLB 200 mg cartridges (Waters, Milford, MA). The eluated fractions were evaporated and the residues were reconstituted with MeOH (0.4 mL) filtered through 0.20 mm syringe polyvinylidene difluouride (PVDF) filters and injected. Quantitative online high-performance liquid chromatography–electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) analyses were performed using an HPLC system interfaced to an Applied Biosystems (Foster City, CA, USA) API3200 Q-Trap instrument working with triple quadrupole analyser in a multiple reaction monitoring (MRM) mode. Latent class analyses were conducted using a system equipped with a 200 binary pump (Perkin-Elmer, USA). Samples were injected (10 mL) into an Atlantis column (Waters) (150 × 2.1 mm

Table 1
Phenolea Active Complex Composition

Specification of Phenolea® Active Complex as indicated by the manufacturer		
Appearance		Red rubin molasses
Solubility in water	%	>99
Microbiological		
Salmonellae SPP:		absent in 25 g
Escherichia coli		absent in 1 g
Yeast and moulds	CFU/g	≤5 × 102
Enterobacteria	CFU/g	≤1 × 102
Total plate count	CFU/g	≤5 × 105
Pesticides		absent
Aflatoxins		absent
Ochratoxin		absent
Polycyclic aromatic hydrocarbons	μg/kg	<1
Chemical composition of Phenolea® Active Complex		
Moisture	%	28
Carbohydrates	%	61
Ashes	%	6.5
Proteins	%	2.5
Fats	%	0
Crude fiber	%	2
Phenolic composition of Phenolea® Active Complex		
Total polyphenols	%	5
Phenolic families (% on total polyphenols)		
Hydroxytyrosol	%	30
Other hydroxyyrosol derivatives	%	20
Ligstroside and derivatives	%	6
Total secoiridoid acids	%	14
Total phenolic acids	%	10
Oleocanthal	%	2
Other polyphenols	%	18