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Protective effect of olive leaf extract on hippocampal injury induced by transient global cerebral ischemia and reperfusion in Mongolian gerbils

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ABSTRACT

The beneficial effects of antioxidant nutrients, as well as complex plant extracts, in cerebral ischemia/reperfusion brain injury are well known. Mediterranean diet, rich in olive products, is associated with lower incidence of cardiovascular disease, cancer, inflammation and stroke. In this study, the possible neuroprotective effect of standardized dry olive leaf extract (OLE) is investigated for the first time. Transient global cerebral ischemia in Mongolian gerbils was used to investigate the OLE effects on different parameters of oxidative stress and neuronal damage in hippocampus. The biochemical measurements took place at different time points (80 min, 2, 4 and 24 h) after reperfusion. The effects of applied OLE were compared with effects of quercetin, a known neuroprotective plant flavonoid. Pretreatment with OLE (100 mg/kg, *per os*) significantly inhibited production of superoxide and nitric oxide, decreased lipid peroxidation, and increased superoxide dismutase activity in all time points examined. Furthermore, OLE offered histological improvement as seen by decreasing neuronal damage in CA1 region of hippocampus. The effects of applied OLE were significantly higher than effects of quercetin (100 mg/kg, *per os*). Our results indicate that OLE exerts a potent neuroprotective activity against neuronal damage in hippocampus after transient global cerebral ischemia, which could be attributed to its antioxidative properties.

Introduction

Mediterranean diet, rich in olive drupes and olive oil, is associated with the lower incidence of cardiovascular disease, cancer, inflammation and stroke (Trichopoulou and Critselis 2004; Fung et al. 2009). Olive (*Olea europaea* L.) phenolics are powerful antioxidants, both *in vitro* and *in vivo* and it is known that olive oil represents a key healthy component of Mediterranean diet (Fitó et al. 2007). Moreover, it has been reported that dietary intake of virgin olive oil, thanks to its *minor* constituents, shows neuroprotective effects (González-Correa et al. 2007).

Not only olive oil, but olive leaf also has different beneficial effects on human health (El and Karakaya 2009). The main constituent of the olive leaf is oleuropeine, one of iridoide monoterpenes, which is thought to be responsible for its pharmacological effects. Furthermore, the olive leaf contains triterpenes (oleanolic and maslinic acid), flavonoides (luteolin, apigenine, rutin), and chalcones (olivin, olivin-diglucoside). It has been traditionally used in hypertonia, arteriosclerosis, rheumatism, gout, diabetes mellitus, and fever (Fleming 2000). A number of papers have been published reporting different pharmacological effects of olive leaf and its constituents, but none of them has focused on neuroprotective activity of total olive leaf extract (OLE) in an global ischemia animal model.

Cerebral ischemia and reperfusion (I/R) is known to induce the generation of reactive oxygen species (ROS), which, in turn, leads to oxidative damage of membrane lipids, proteins and nucleic acids (Chan 2001). In global cerebral ischemia, increased production of ROS has been regarded as an underlying factor for mediating delayed neuronal death, especially to pyramidal neurons in the hippocampal CA1 area (Hara et al. 2000). This has raised attention to testing possible beneficial effects of different antioxidants, especially those from plant sources, on cerebral injury after stroke. The beneficial effects of antioxidant nutrients in cerebral ischemia and recirculation brain injury are previously reported (Ikeda et al. 2003). Neuroprotective effects of single phenolics, such as resveratrol from grape and red wine, curcumin from turmeric, apocynin from *Picrorhiza kurroa*, and epi-gallocatechin from green tea were evaluated (Sun et al. 2008). However, the reports of neuroprotection by natural compounds from plants frequently refer to complex

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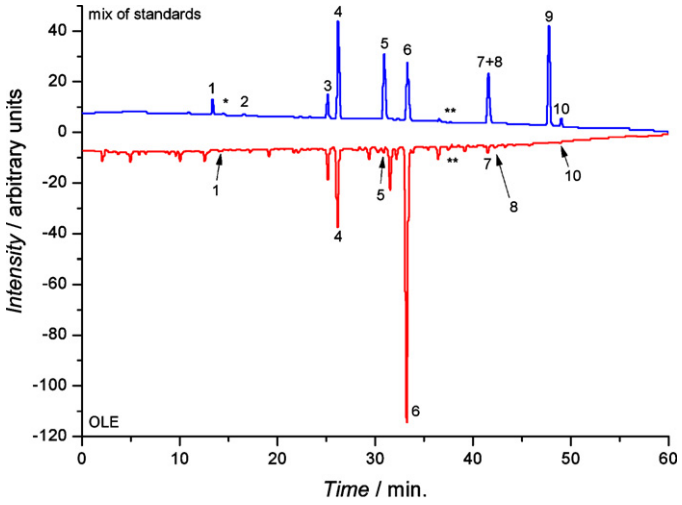

Fig. 1. HPLC chromatogram of the olive leaf extract recorded at 260 nm and compared to the standard mix of identified compounds. The numbers refer to the following: 1, caffeic acid; 2, vanilin; 3, rutin; 4, luteolin-7-*O*-glucoside; 5, apigenin-7-*O*-glucoside; 6, oleuropein; 7, quercetin; 8, luteolin; 9, apigenin; and 10, chryseriol. *Impurity from caffeic acid; **impurity from the oleuropein standard. Reproduced from Dekanski et al. (2009) with permission.

extracts like those of *Ginkgo biloba* (Lee et al. 2002) and green tea (Hong et al. 2001) and not to a single compounds.

In light of the above considerations and since OLE is known for its anti-oxidative activity (El and Karakaya 2009), we investigated, for the first time, the possible neuroprotective effect of total OLE in the hippocampus of Mongolian gerbils subjected to transient global cerebral I/R.

Materials and methods

Chemicals

Olive leaf extract EFLA® 943, standardized to 18–26% of oleuropein, was purchased from Frutarom Switzerland Ltd. (Wadenswil, Switzerland). The extract was manufactured from the dried leaves of *Olea europaea* L., applying an ethanol (80% m/m) extraction procedure. After a patented filtration process (EFLA® Hyperpure), the crude extract was dried. Stability and microbiological purity were confirmed by the manufacturer. The extract was further analyzed in our previous study, and it was detected that its total phenols content, determined by Folin-Ciocalteau assay, was 197.8 μg GAE *per* g of dry extract; total flavonoids and tannins content was 0.29% and 0.52%, respectively. HPLC analysis (Fig. 1 and Table 1) revealed a complex mixture of phenolic compounds: oleuropein, luteolin-7-*O*-glucoside, apigenine-7-*O*-glucoside, quercetin and caffeic acid (Dekanski et al. 2009). In this study, the same batch of EFLA® 943 is used. Quercetin, butylated hydroxytoluene (BHT) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) were purchased from Sigma (St. Louis, MO, USA), PEG 400 from BASF, Germany. All other chemicals used for biochemical analyses were from Sigma.

Determination of antioxidant activity in vitro

Antioxidant capacity of the both olive leaf extract and quercetin was measured using the DPPH assay based on the scavenging ability to 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical (Goupy et al. 1999). Butylated hydroxytoluene (BHT) was used as a positive control. Briefly, the samples in different concentrations were mixed with DPPH solution and ethanol. After vortexing, the tubes were left in the dark at room temperature after which the absorbance was measured at 517 nm using a UV–vis spectrophotometer HP 8453 (Agilent Technologies, Santa Clara, CA). Each measurement was performed in triplicate under identical conditions. Antioxidant activities were expressed as the IC_{50} values, i.e., the concentration of antioxidant required to cause 50% reduction in the original concentration of DPPH.

Table 1
Quantitative determination of flavonoids, phenolcarbonic acids and oleuropein in olive leaf extract.

Compound name[a]	Amount	
	mg	%
Caffeic acid (1)	0.013	0.02
Vanilin (2)	Not found	
Rutin (3)	Not found	
Luteolin-7-*O*-glucoside (4)	0.027	0.04
Apigenin-7-*O*-glucoside (5)	0.046	0.07
Oleuropein (6)	13.147	19.8
Quercetin (7)	0.027	0.04
Luteolin (8)	Trace[b]	
Apigenin (9)	Not found	
Chryseriol (10)	Trace[b]	

Reproduced from Dekanski et al. (2009) with permission.
[a] The numbers refer to the compounds marked on the HPLC chromatogram (Fig. 1).
[b] Determination was not possible – present in the extract under the limit of quantitative analysis.

Experimental animals

Adult male Mongolian gerbils (*Meriones unguiculatus*, 55–65 g) were used in this study. Groups of four gerbils per cage (Erath, FRG), were housed in an air-conditioned room, at the temperature of 23 ± 2 °C, with 55 ± 10% humidity, and with lights on 12 h/day (7:00–19:00). The gerbils were given commercial food and tap water *ad libitum*. All experimental manipulations were performed during the light phase, between 9:00 and 15:00, under identical conditions. Animals used for procedures were treated in strict accordance with the NIH Guide for Care and Use of Laboratory Animals (1985) and the European Communities Council Directive (86/609/EEC), as well as with approval of the local Ethical Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Occlusion of common carotid arteries

Global ischemia occurs when cerebral blood flow is reduced throughout the most part or the entire brain. Since mature gerbils lack posterior communicating arteries, those normally connect the posterior circulation of the brain from the vertebral arteries with the anterior circulation from the carotid arteries within the circle of Willis, occlusion of both common carotid arteries results, reproducibly, in global forebrain ischemia. The Mongolian gerbils were anaesthetized by diethyl ether and placed in the dorsal position. The neck area was shaved, and then both common carotid arteries were exposed carefully by blunt dissection and clamped for 10 min with microaneurysm clips. After the clips were removed, reperfusion was confirmed visually, and the skin was sutured by 3–4 loose silk stitches. For sham-operated animals, both common carotid arteries were exposed but not occluded. Post-ischemic temperature was carefully monitored. Since the changes in body temperature are known to have impact on the consequences of global ischemia, it was maintained at 37 ± 0.3 °C throughout the surgical procedure by a feedback-controlled heating pad (TR-100, PS-100, Fine Science Tool, North Vancouver, Canada). Gerbils were allowed to recover in their home cages for 2 h under a Xenon heating lamp and then returned to animal quarters.