Protective Role of Oleuropein against Acute Deltamethrin-Induced Neurotoxicity in Rat Brain

Ali Reza Khalatbary*1, Elmira Ghaffari2 and Behrooz Mohammadnegad2

1Molecular and Cell Biology Research Center, Dept. of Anatomy, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran; 2Dept. of Anatomy, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Received 27 December 2014; revised 6 April 2015 ; accepted 14 April 2015

ABSTRACT

Background: Deltamethrin (DM) is a synthetic pyrethroid insecticide that can elicit neurotoxicity, leading to apoptosis. There is accumulating evidence that oleuropein (OE) has anti-apoptotic effect. The purpose of this study was to determine the anti-apoptotic effect of OE pretreatment in the neuronal cells of cerebral cortex. Methods: Rats were randomly divided into four groups each containing five rats: DM-treated group (12.5 mg/kg, a single dose), OE-treated group (20 mg/kg per day), DM + OE-treated group, and vehicle group. Sections of the brain were obtained 24 hours after DM injection and studied for histopathological and immunohistochemistry assessment. Results: The histopathological assessments showed lesser characteristics of neural degeneration in DM + OE group compared with DM group. Greater Bcl-2 and attenuated Bax expression could be detected in the DM + OE treated-mice compared with DM group. Conclusion: The results suggested that DM-induced neurotoxicity can be subsided by OE. Iran. Biomed. J. 19 (4): 247-253, 2015

Keywords: Deltamethrin, Oleuropein, Apoptosis

INTRODUCTION

Deltamethrin (DM) is a type II synthetic pyrethroid insecticide used as a major class of insecticides in agriculture worldwide [1]. Acute exposure to DM can elicit neurotoxicity and can be characterized by ataxia, loss of coordination, hyperexcitation, convulsions, and paralysis [2]. Neurotoxicity of DM is mediated by a series of cellular, molecular, and biochemical cascades, including the modification of sodium channels kinetics [3], increasing neurotransmitter release [4], S100β upregulation [5], induction of oxidative damage [6], and induction of cytochrome P450s [7]. Moreover, in vitro and in vivo studies have suggested the important role of apoptosis in neurotoxicity of DM [8, 9]. Apoptosis or programmed cell death is a key mechanism of neurodegenerative diseases, which is triggered by toxins, radiation, hypoxia, oxidative stress, ischemia/reperfusion, loss of survival/trophic factors, and DNA damage [10]. A number of studies have revealed that exposure to DM significantly affects the survival of neurons in rat brain and induces mitochondria-mediated apoptosis [11, 12]. Each treatment, which interrupts the apoptosis processes, could improve the DM neurotoxicity. Within the previous decades, a rapidly growing number of natural polyphenol compounds have been described to have anti-apoptotic effects. One of the main sources of these molecules is olive oil. Olive oil is a rich source of polyphenolic components similar to its main component oleuropein (OE, 3, 4 dihydroxy-phenylelenolic acid), which have many beneficial health effects on human [13-15]. There is accumulating evidence that has attributed the beneficial effects of OE and its derivatives to a variety of biological activities, including free radical scavenging/antioxidant actions, anti-inflammatory effects, and anti-carcinogenic as well as anti-apoptotic properties [15, 16]. In this regard, some experimental studies have documented that OE and its derivatives have anti-apoptotic effects on intestinal ischemia/reperfusion injury [17], 6-...
hydroxydopamine-induced PC12 cell apoptosis [18],
and doxorubicin-induced cardiomyopathy [19].
Accordingly, in this work, we evaluated the effect of
OE on the activity and the expression of apoptotic
criteria against acute DM-induced neurotoxicity in rat
brain.

MATERIALS AND METHODS

Animals. Female adult Sprague–Dawley rats (180–
200 g, Pasteur Institute, Tehran, Iran) were used in this
study. The animals were kept under standard
conditions and fed a standard rat chow and drinking
water ad libitum throughout the study period. DN and
OE were purchased from Sigma (Germany). The rats
were randomly allocated into four groups, each
containing 5 rats: (1) DM-treated group (a single
intraperitoneal dose of 12.5 mg/kg) [12]; (2) OE-
treated group (intraperitoneally at a dose of 20 mg/kg
per day for 7 days) [20]; (3) DM + OE-treated group
was given pretreatment of OE for 7 days at 20 mg/kg
per day with a single intraperitoneal dose of 12.5
mg/kg DM on the seventh day; (4) vehicle group.

Histopathological assessment. Brain samples were
obtained 24 hours after DM injection, fixed in 10%
wt./vol.) PBS-buffered formaldehyde and embedded
in paraffin. The coronal sections (5 µm) of frontal
cortex were selected randomly using a microtome. For
histopathological assessment, some tissue sections
were deparaffinized with xylene, stained with
hematoxylin-eosin (H & E) and cresyl violet, and
studied using light microscopy (DME; Leica
Microsystems Inc., Buffalo, NY, USA). All the
histological studies were performed in a blinded
fashion.

Immunohistochemistry. For immunohistochemistry,
the sections of frontal cortex were incubated in the goat
serum (in order to block nonspecific site), polyclonal
rabbit anti-Bax antibody (1:50 in PBS, vol./vol.,
Abcam, USA), or anti-Bcl-2 rabbit polyclonal antibody
(1:100 in PBS, vol./vol., Abcam, USA) at 4°C
overnight. The sections were then washed with PBS
and incubated with secondary antibody conjugated
with horseradish peroxidase (goat anti-rabbit IgG,
Abcam, USA) for 2 hours and detected by
diaminobenzidine tetrahydrochloride for 5 minutes.
Afterwards, they were dehydrated and mounted. For
negative controls, primary antibodies were omitted.
For quantitative analysis, immunohistochemical
photographs (5 photos from each samples collected
from all rats in each experimental group) were assessed
by densitometry using MacBiophotonics Image J 1.41a
software on an ASUS personal computer.

Statistical analysis. Statistical analysis was carried
out using the SPSS package (version 15, Chicago, IL,
USA)., and the results were presented as mean values
(±SD). The K-S test was used to evaluate the normality
of the data. Also, the Tukey's multiple comparison test
and the analysis of the variance were used to compare
each of the two groups as well as compare the data
among the groups, respectively. A value of P < 0.05
was considered statistically significant.

RESULTS

Histopathological assessments. To observe the
morphological characteristics of cortical neurons in rat
brain of all experimental groups, the H & E and cresyl
violet staining were used in the present study. Histopathological study with H & E staining showed
that some degenerative changes in cortical neurons
(pyknosis of nuclei and shrinkage of cytoplasm) (Fig.
1A), and with cresyl violet staining, it indicated
shrinkage and strong staining of Nissl bodies in the
brain of DM-treated rats (Fig. 2A). However, little or
no signs of degeneration were seen in OE-treated (Fig.
1B and 2B) and DM + OE-treated groups (Fig. 1C and
2C) or in the vehicle group (Fig. 1D and 2D).

Immunohistochemistry. Figure 3 shows the
immunohistochemical staining of Bax in all groups. Cortical neurons of the brain from OE (0.71 ± 0.05)
(Fig. 3A) and vehicle-treated rats (0.63 ± 0.03) (Fig.
3D) indicated a weak positive immunoreaction for Bax,
whereas the sections of DM-treated rats exhibited a
strong positive staining for Bax (9.64 ± 2.19) (Fig. 3B).
OE treatment in DM + OE-treated rats reduced the
degree of positive staining for Bax (1.64 ± 0.28) (Fig.
3C). Figure 4 shows the immunohistochemical staining
of Bcl-2 in all groups. The expression of Bcl-2 was
strong in cortical neurons of the brain from the OE-
(9.52 ± 1.93) (Fig. 4A) and vehicle-treated rats (9.59 ±
2.20) (Fig. 4D). In contrast, it was weak in the DM-
treated rats (0.83 ± 0.11) (Fig. 4B) compared to the up-
regulation in the DM + OE-treated rats (5.12 ± 0.80)
(Fig. 4C).

Quantitative analysis. The histograms of the
quantitative analysis of Bax and Bcl-2 staining in the
experimental groups are shown in Figures 5 and 6,
respectively.

DISCUSSION

Neurotoxins are well known risk factors for chronic
neurodegenerative diseases. Although molecular
mechanisms involved in the pathogenesis of
Fig. 1. Hematoxylin-eosin staining of paraffin sections from the brain of DM (A), OE (B), DM + OE (C), and vehicle (D) treated rats. Many neuronal cells of cerebral cortex showed characteristics of degeneration with pyknosis of nuclei and shrinkage of cytoplasm in DM group, ×400. Little or no signs of degeneration were seen in OE, DM + OE, and vehicle groups, ×400. Arrows show DM and vehicle groups.

Fig. 2. Cresyl violet staining of paraffin sections from the brain of DM (A), OE (B), DM + OE (C), and vehicle (D) treated rats. Many neuronal cells of cerebral cortex showed characteristics of degeneration with shrinkage and strong staining of Nissl bodies in DM group, ×400. Little or no signs of degeneration were seen in OE, DM + OE, and vehicle groups, ×400. Arrows show DM and vehicle groups.

Diseases remain unclear, oxidative stress, excitotoxicity, inflammation, and apoptosis have been implicated as possible causes on neurodegeneration [21]. Apoptosis is a key molecular mechanism of neurodegenerative diseases that is regulated by the Bcl-2 family proteins [21]. Among these proteins, Bel-2 and Bax play anti-apoptotic and pro-apoptotic roles, respectively [22]. The ratio of Bax to Bcl-2 determines the cell fate; excess Bcl-2 leads to the survival of cells, while Bax induces apoptosis [23, 24]. In vitro and in vivo studies have shown that apoptosis is a key mechanism of DM neurotoxicity that is mediated by altered expression of P53, Bax, Bcl-2, and caspases [9, 13, 25]. Caspase is a family of cysteine proteases that play essential roles in apoptosis neurodegenerative [26]. P53 is a tumor suppressor gene that can activate...
Fig. 3. Light photomicrographs showing immunohistochemical expression of Bax in OE (A), DM (B), DM + OE (C), and vehicle (D) treated groups (magnification × 400). The positive staining of Bax is presented by the brown color of cytoplasm (arrows).

or repress transcription as well as induce apoptosis [27]. Chen et al. [11] demonstrated that DM may have an effect on mitochondria-mediated apoptosis of nerve cells in rat brain by altered expression of cytochrome c. The cytochrome complex is a small heme protein, which is involved in initiation of apoptosis [28]. DM causes apoptosis through its interaction with Na+ channels, leading to calcium overload and activation of the ER stress pathway [9]. Results of our immunohistochemical assessment showed that the treatment with DM increased positive staining for Bax, whereas exhibited a decreased positive staining for Bcl-2 in the neuronal cells of cerebral cortex of DM group. To date, the majority of epidemiological studies involving olive oil is linked to a decreased incidence of certain types of neurodegenerative diseases such as Alzheimer’s [29], multiple sclerosis [30], and aging [31]. Animal and human studies have demonstrated that olive oil

Fig. 4. Light photomicrographs show immunohistochemical expression of Bcl-2 in OE (A), DM (B), DM + OE (C), and vehicle (D) treated groups (magnification × 400). The positive staining of Bcl-2 is presented by the brown color of cytoplasm (arrows).

http://IBJ.pasteur.ac.ir
Fig. 5. Densitometry analysis of immunohistochemical photomicrographs for Bax. Data are expressed as the percentage of total tissue area. *$p < 0.001$ versus DM group; $^\#p > 0.05$ versus OE and vehicle groups. Bars indicate the standard deviations of the mean (SEM).

Phenolic compounds are highly bioavailable. In this regard, a recent study has shown that after a single ingestion of olive oil phenolic compounds, they were absorbed, metabolized and distributed through the blood stream to practically all parts of the body of rat, even across the blood-brain barrier [32]. On the other hand, in vitro studies have suggested that anti-apoptotic properties of OE and its derivatives are potential neuroprotective mechanisms against neurodegenerative diseases [33]. Results of our immunohistochemical assessment showed that the treatment with OE reduced positive staining for Bax, while on the contrary, it increased positive staining for Bcl-2 in the DM + OE-treated group, thereby provided the molecular evidence for the neuroprotective activity of OE. In this regard, González-Correa et al. [34] documented that lactate dehydrogenase efflux, as a marker of brain cell death, inhibited in a concentration-dependent manner after 7 days of oral treatment with hydroxytyrosol in rat brain slices subjected to hypoxia-reoxygenation. An in vitro study has also indicated that the olive oil phenolic extract and one of its constituents, gallic acid, which exerts anti-apoptotic effect against $H_2O_2$-induced apoptotic cell death in Hela cells with reduction of time-dependent caspase 9 activity [35]. Furthermore, another study documented that the incubation of PC12 cells with OE could decrease cell damage and reduce biochemical markers of apoptotic cell death including activated caspase 3, Bax/Bcl-2 ratio, and DNA fragmentation in 6-hydroxydopamine-induced PC12 cell apoptosis [19]. Histological and molecular examinations demonstrated that OE aglycone modulated apoptosis pathway, as shown by tunel staining and Bax/Bcl-2 expressions in a murine model of intestinal ischemia/reperfusion injury [18]. A recent study has shown that OE prevents doxorubicin-induced cardiomyopathy through the modulation of kinases such as Akt [20], a serine/threonine-specific protein kinase that plays a key role in apoptosis and cell proliferation [36].

In the present study, it is clear that DM exposure resulted in alternations of Bax/Bcl-2 expressions and apoptosis in the neuronal cells of cerebral cortex, while OE pre-exposure provided protection against DM-induced apoptosis in terms of histopathological and immunohistochemical expression of the pro- and anti-apoptotic protein. In conclusion, this study suggests that OE has modulatory effects on DM-induced apoptosis in the neuronal cells of rat cerebral cortex.

ACKNOWLEDGEMENTS

This work was supported financially by Molecular and Cell Biology Research Center, Faculty of Medicine, Mazandaran University of Medical Sciences.

REFERENCES


34. González-Correa JA, Navas MD, Lopez-Villodres JA,
