Toxicology in Vitro 52 (2018) 279–285

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Long-term neurotoxic effects of domoic acid on primary dopaminergic neurons

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ARTICLE INFO

Keywords:
Domoic acid
Dopaminergic neurons
Parkinson's disease
Neurotoxicity
Marine
In vitro
AMPA
KA

ABSTRACT

Domoic acid, an excitatory neurotoxin produced by certain algae, reaches the food chain through accumulation in some sea organisms. To investigate its long-term neurotoxicity on dopaminergic neurons, prepared primary mesencephalic cell cultures were exposed to different concentrations of domoic acid (0.1, 1, 10, 100 μM) on the 8th day in vitro (DIV) for 4 days. On the 12th DIV, culture media were collected for measurement of lactate dehydrogenase and cultured cells were subjected to immunohistochemistry against tyrosine hydroxylase, neuronal nuclear antigen and glial fibrillary acidic protein, and fluorescence staining using H_2DCFDA, JC-1 and Hoechst 33342 dyes. Moreover, roles of AMPA/KA and NMDA receptors in domoic acid neurotoxicity were also investigated. Domoic acid significantly decreased the number of dopaminergic neurons and adversely affected their morphology, and slightly reduced the expression of neuronal nuclear antigen and glial fibrillary acidic protein. Co-treatment of cultures with domoic acid and the AMPA/KA or NMDA receptor antagonists NBQX and MK-801 rescued significant number of dopaminergic neurons. Domoic acid significantly decreased red:green fluorescence ratio of JC-1 and did not affect production of reactive oxygen species and apoptotic cell death. In conclusions, the present study reveals that long-term treatment of primary mesencephalic cell culture with domoic acid significantly destroyed dopaminergic neurons. This effect appears to be attributed to activation of AMPA/KA and NMDA receptors and mitochondrial damage.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting about 2–3% of people older than 65 years. The neuropathological hallmarks of the disease are the loss of dopaminergic neurons in substantia nigra (SN), which leads to striatal dopaminergic deficiency, and formation of intracellular inclusions containing α-synuclein aggregates in the surviving neurons (Poewe et al., 2017). As a movement disorder, PD disease is characterized by a group of cardinal signs, most notably resting tremors, rigidity and bradykinesia (Cacabelos, 2017). Moreover, PD is associated with a group of non-motor symptoms as the pathological process affects multiple other cell types in the central and peripheral nervous system (Poewe et al., 2017). Although the exact etiology of PD is still unknown, numerous epidemiological studies link the higher PD risk to some environmental factors including consumption of well water, living in rural areas, and exposure to herbicides and pesticides (Ritz et al., 2016; Naughton et al., 2017; Rokad et al., 2017). In addition to the interaction with the genetic agents, environmental factors play an important role in mitochondrial dysfunction, oxidative stress and modifying proteasomal function (Schapira, 2006).

Domoic acid (DomA) is a potent marine neurotoxin that naturally produced by some marine organisms such as the red alga *Chondria armata* and planktonic diatom of the genus Pseudonitzchia (Hiolski et al., 2016). When DomA is produced at high concentrations in diatoms, it can bioaccumulate in some marine organisms such as shellfish, sardines and anchovies, causing the poisoning of seabirds, marine mammals or humans (Lefebvre and Robertson, 2010). In 1987, DomA caused four mortalities and illness of > 100 people after consuming the blue mussels *Mytilus edulis* in the Cardigan Bay of Prince Edward Island, Canada (Perl et al., 1990). Symptoms in ill people comprised three kinds of signs: (1) gastrointestinal (nausea, vomiting, abdominal cramps and diarrhea), (2) cardiovascular (arrhythmias and unstable blood pressure)

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https://doi.org/10.1016/j.tiv.2018.07.004
Received 10 March 2018; Received in revised form 9 July 2018; Accepted 10 July 2018
Available online 11 July 2018
0887-2333/

and (3) neurological signs (disorientation, headache, hallucination, seizures, memory impairment and coma). Impairment of memory was the reason to denominate this condition as amnesic shellfish poisoning (ASP) (Addison and Stewart, 1989; Wright et al., 1989). Since that time, specific control measures have been implemented in Canada and all over the world to prevent food-borne illness due to DomA (Park, 1995; Toyofuku, 2006). However these measures have been successful in preventing other episodes of ASP, some DomA intoxication have been reported in wild animals including sea lions, whales, sea otters and sea birds (Sierra et al., 1997; Lefebvre et al., 1999; Gulland et al., 2002; Lefebvre et al., 2002; Goldstein et al., 2008).

Neurotoxicity of DomA was reported in some in vitro and in vivo experimental models. For instance, Giordano et al. (2008) and Ramya et al. (2017) found that DomA significantly produced apoptotic cell death in cerebellar granule neurons (CGNs) and Caco-2 cell line. Strain and Tasker (1991) reported that intraperitoneal injection of DomA produced a series of behavioral changes in mice including sedation, rigidity, stereotypy, balance loss and convulsions. Pathologically, the authors observed that DomA resulted in hippocampal neuronal damage particularly in the CA3 region (Strain and Tasker, 1991).

As to date there have been no studies showed the neurotoxic effect of DomA on dopaminergic neurons, the current study was conducted to investigate the long-term neurotoxicity of DomA on dopaminergic neurons in primary mesencephalic cell culture and whether it could be implicated in PD.

2. Materials and methods

2.1. Preparation of primary mesencephalic cell cultures

Pregnant mice (OF1/SPF, Himberg, Austria) were cared and handled in accordance with the guidelines of the European Union Council (86/609/EU) for the use of laboratory animals. At gestation day 14, primary mesencephalic cell cultures were prepared from embryonic mesencephala as described previously by Radad et al. (2015). The embryos were collected at gestation day 14 under aseptic condition in Dulbecco's phosphate buffered saline (DPBS, Invitrogen, Germany). Under a stereoscope, the brains were released and the mesencephala were excised, carefully cleaned from meninges, and enzymatically and mechanically dissociated using 0.2% trypsin solution (Invitrogen, Germany) and fire-polished Pasteur pipettes, respectively. Then, obtained cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 mM HEPES buffer, 4 mM glutamine and 10% heat-inactivated fetal calf serum (FCS). The medium was exchanged with DMEM supplemented with FCS on the 1st and 3rd DIV. On the 5th DIV, half of the medium was replaced with serum-free DMEM containing 0.02 ml B-27/ml (Invitrogen, Germany). Serum-free DMEM supplemented with 0.02 ml B-27/ml was used for feeding of cultured cells from the 6th DIV and subsequently replaced every 2nd day.

2.2. Treatment of cultures with DomA

DomA (Sigma, Germany) was prepared in a stock solution of 1 mM in distilled water. Final concentrations of DomA were prepared in DMEM. On the 8th DIV, cultures were treated with different concentrations of DomA (0.1, 1, 10, 100 µM) for 4 days. Culture media were changed with the same concentrations of DomA on the 10th DIV. On the 12th DIV, culture media were obtained for measurement of lactate dehydrogenase (LDH) and cultured cells were subjected to immunohistochemistry against tyrosine hydroxylase (TH), neuronal nuclear antigen (NeuN) and glial fibrillary acidic protein (GFAP), and fluorescence staining using H_2DCFDA, JC-1 and Hoechst 33342 dyes.

2.3. Identification of dopaminergic neurons

Dopaminergic neurons were visualized in primary mesencephalic cell cultures by immunostaining against TH. Cultured cells were fixed with histochoice for 15 min at room temperature. Then, they were permeabilized with 0.4% Triton X-100 for 30 min at room temperature. After blocking of non-specific binding sites with 5% horse serum (Vectastain ABC Elite kite) for 90 min at room temperature, cells were incubated with anti-TH primary antibody overnight at 4°C, biotinylated secondary antibody (Vectastain) and avidin-biotin-horseradish peroxidase complex (Vectastain) for 90 min at room temperature. The reaction product was developed in a solution of diaminobenzidine (1.4 mM) in PBS containing 3.3 mM hydrogen peroxide (H_2O_2). Dopaminergic neurons were counted with a Nikon inverted microscope in 10 randomly selected fields/well at 10× magnification.

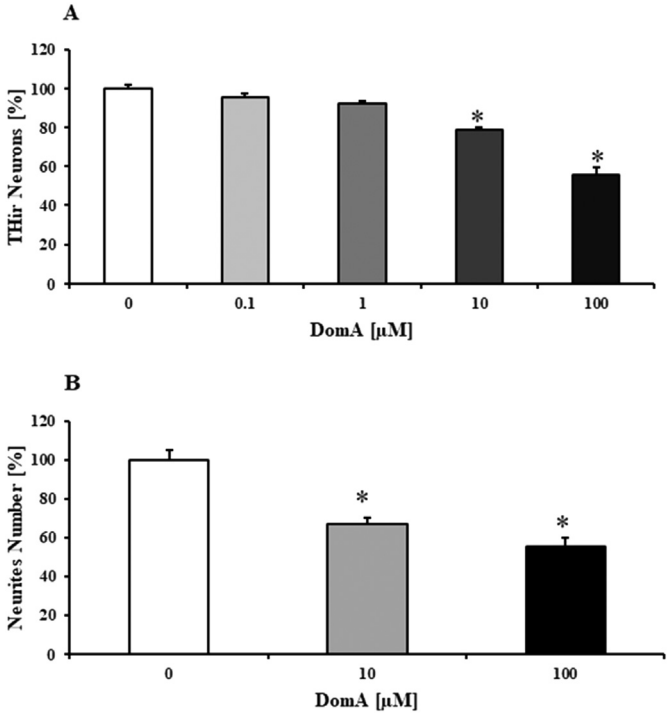

Fig. 1. Effect of DomA on dopaminergic neurons in primary mesencephalic cell culture. A) DomA significantly decreased the number of dopaminergic neurons at the concentrations 10 µM (78.80 ± 1.5) and 100 µM (55.43 ± 3.94) compared to untreated controls. B) Also, DomA significantly decreased the number of dopaminergic neurites at 10 µM (66.67 ± 3.46) and 100 µM (55.17 ± 4.76) compared to untreated controls. 100% corresponds to the total number of dopaminergic neurons (the average number was 20.75 cells/field) and dopaminergic neurites (the average number was 3/cell) after 12 DIV in untreated control cultures. (*$p < 0.001$).

2.4. Detection the effects of DomA on total neuronal cells and astrocytes

Two sets of cultures were treated with DomA (10 and 100 µM) on the 8th DIV for 4 days. Culture media were changed with the same concentrations of DomA on the 10th DIV. On the 12th DIV, cultures were stained immunocytochemically using anti-NeuN and anti-GFAP antibodies (Chemicon, USA) for visualizing postmitotic neuronal cell types and astrocytes, respectively. In which, the same staining procedures were carried out as described for anti-TH immunostaining except that the anti-TH antibody was replaced with the anti-NeuN or anti-GFAP antibodies. NeuN immunoreactivity was quantitatively assessed in 5 photographs/treatment condition using Adobe Photoshop software®. Briefly, the original photographs were converted into the black-white by the threshold tool. Then, the black color that corresponds to the number of NeuN+ neurons were measured and expressed as the percentage of the averaged control. Astrocytes were counted with a Nikon inverted microscope in 10 randomly selected fields/well at 40×