The protective effect of docosahexaenoic acid on the stomach in Parkinson's disease induced by MPTP in male C57BL/6 mice

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ABSTRACT

The purpose of this study was to detect gastric changes in Parkinson's disease induced by 1-methyl-4-phenyl-1.2.3.6.-tetrahydropyridine (MPTP), and to investigate the protective effect of docosahexaenoic acid (DHA) against these changes in mice. The mice were divided into 4 groups (n=10 in per group) as control, DHA, Parkinson and DHA+Parkinson groups. DHA was administered by gavage for 30 days. On the 23rd day of gavage treatment, the animals of the Parkinson and DHA+Parkinson groups were intraperitoneally injected with MPTP. Seven days after the injection of MPTP, their locomotor activity, bradykinesia and rotarod performance were measured. Tyrosine hydroxylase expression in substantia nigra and the apoptotic index, the concentrations of tumor necrosis factor-α and histamine, and the number of mast cells in the stomach were evaluated. Administration of DHA significantly prevented the reduction in motor functions ($P<0.001$) and nigral TH neurons ($P<0.05$), and apoptosis ($P<0.05$), and an increase in TNF-α concentration ($P<0.01$) in the stomach. An increase in the number of mast cells in the stomach wall was observed in PD ($P<0.001$). DHA prevented the increase in the number of mast cells ($P<0.05$) and the histamine level ($P<0.01$) due to PD. As a result, MPTP administration in mice caused changes in the stomach as well as impairment in motor functions, and DHA was observed to reduce these changes.

Key words: docosahexaenoic acid; gastric tissue; MPTP; apoptosis; mast cells; mice

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease, affecting approximately 2% of individuals over 65 and 10% of individuals over 80 years of age (FORMAN et al., 2004). Although

PD is known as a central nervous system (CNS) disease caused by the loss of dopaminergic neurons projecting from the substance nigra (SN) to the striatum, it has been accepted as a systemic disease in recent years and also affects peripheral tissues

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(EZQUERRA et al., 2019). The characteristic clinical motor symptoms of PD are bradykinesia, resting tremor and rigidity. These findings are due to the loss of approximately 70% of dopaminergic neurons in the pars compact segment of the SN (LEGGIO et al., 2017). PD also has non-motor symptoms such as cognitive disorders, hyposmia, pain, depression, sleep problems, autonomic dysfunction and gastrointestinal (GI) dysfunction, due to the loss of neurons in the periphery and CNS (CHAUDHURI et al., 2005). Importantly, especially GI symptoms, such as impaired gastric emptying, gastric ulcer and bowel dysfunction, are observed before the motor symptoms of PD (ABBOTT et al., 2001). Furthermore, these dysfunctions in the gastrointestinal system (GIS) contribute directly to the morbidity of PD. The factors responsible for the neuron loss, leading to the onset and progression of the pathological process in PD are not fully understood. However, in addition to genetic factors, neuroinflammation triggered by environmental factors, activation of glial cells, misfolding of proteins, α-synuclein accumulation in cells, mitochondrial dysfunction, decreased antioxidant capacity, increased oxidative stress and proinflammatory molecules are defined as the key mechanisms that cause PD (GAUTIER et al., 2014). In the pathological process in the CNS, mast cells have been especially emphasized in recent years. Mast cells, one of the proinflammatory cytokine sources, affect the neurons and microglia, and contribute to neurodegeneration (JONES et al., 2019).

The relationship between PD and GI symptoms has received increased attention in recent years (CERSOSIMO et al., 2013), since there is a bilateral neural communication between the central dopaminergic system and the GIS. Loss of dopaminergic neurons in the SN causes changes in neurotransmitter release in the GIS, leading to GI disorders, and pathologies in the GIS affect dopamine homeostasis in the SN (ANSELMI et al., 2017; PELLEGRINI et al., 2016). The SN directly/indirectly modulates the dorsal motor nucleus of the vagus (DMV) regulating GI functions (ANSELMI et al., 2017). Therefore, GI disturbances observed in the early stages of PD may

result from the initial loss of nigral dopaminergic neurons. Conversely, mucosal inflammation and oxidative stress caused by various experimental toxins, such as various pathogens and/or pesticides, may cause dopaminergic vulnerability and induce neurodegeneration in the SN (HOLMQVIST et al., 2014). Furthermore, the gut-brain axis, mediated by the vagus, is important for nigrostriatal dopamine homeostasis and gastroprotection (BIRSEN et al., 2020; GARRIDO-GIL et al., 2018). Dysregulation of this neural bidirectional communication is thought to explain the GI disorders observed in early stages of PD (GARRIDO-GIL et al., 2018). Therefore, the activation of antioxidant mechanisms, and modulation of the mast cell responses and inflammatory/apoptotic pathways in periphery or CNS may some important targets to prevent the progression of PD and reduce the symptoms.

Experimental studies have reported that there are beneficial effects from dietary consumption of long-chain polyunsaturated fatty acids (PUFAs) in many systemic pathologies, such as diabetes, cancer, cardiovascular and nervous diseases (GORJAO et al., 2009; SKENDER et al., 2012). As PUFAs such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are essential fatty acids, the most effective way to increase the PUFA content in cells is through dietary supplementation (MARANGONI and POLI, 2013). Recent studies have established a relationship between PUFA supplementation and the incidence of neurodegenerative diseases, such as Alzheimer's disease (WU et al., 2015). DHA (22:6 n-3) is the best represented omega-3 fatty acid, important for the development of neuronal cells (KIM et al., 2001). Furthermore, DHA has anti-apoptotic effects in neuronal cells and increases synaptogenesis. It was shown that DHA inhibits microglial activation and reduces the proinflammatory cytokine release and oxidative stress (CHANG et al., 2015; HUUN et al., 2018). The effect of DHA is mediated at least in part through activating the PI3K/Akt pathway and inhibiting COX-2 expression (AKBAR and KIM, 2002; LEE et al., 2009).

The protective effect of DHA on the dopaminergic neurons in the SN and motor

performance has been shown in previous studies (CHITRE et al., 2020; OZKAN et al., 2016). In PD, a decrease in dopaminergic neurons in the SN is observed due to apoptosis. It has been shown in previous studies that motor dysfunction is due to the loss of dopaminergic neurons. Dopaminergic neurons are also found in the enteric nervous system. It is thought that dysfunction in the GI tract due to PD may be due to the loss of neurons in the enteric nervous system with apoptosis. However, there is no study on this subject. A Parkinson's model induced by MPTP is a commonly used mice model of PD (PRZEDBORSKI et al., 2001). For this reason, the aim of this study was to investigate whether there are apoptotic changes in the stomach due to PD, and to evaluate the use of DHA in prevention of /protection from these changes.

Materials and methods

Animals. Male C57BL/6 mice (weighing 25–30 g) supplied by the Akdeniz University Faculty of Medicine Experimental Animals Care and Production Unit were used for this study. The animals were treated in accordance with the Guide for the Care and Use of Laboratory Animals Committee of Akdeniz University. The mice were accommodated in a temperature-controlled environment (22 ± 1°C) with a 12 h/12 h light/dark cycle, and allowed to access standard laboratory feed and tap water until the experiments. All the animals were healthy during the experimental procedures and there were no differences in body weights and survival rates (Data not shown).

Experimental Procedures. Forty male mice, weighing 25-30 g, provided under the same conditions, were randomly divided into four groups (n=10 per group) as follows: 1) Control group (C); 2) DHA-treated group (DHA); 3) Parkinson group (P) and 4) DHA+Parkinson group (DHA+P). DHA (Sigma-Aldrich, St. Louis, MO, USA) dissolved in corn oil at a concentration of 0.046 M was given to the DHA-treated groups for 30 days (36 mg/kg/day) by gavage (0.1 ml) (HACIOGLU et al., 2006; HACIOGLU et al., 2012). The control and Parkinson groups received a similar volume of corn oil alone. On the 23rd day of the gavage treatment of DHA, MPTP (20 mg/kg bw in 0.6

ml of saline), Sigma, St. Louis, MO, USA) were injected intraperitoneally four times at 2-hour intervals into the P and DHA+P groups (BEAL et al., 1998; DATE et al., 1990). After the behavioral tests, the mice were anesthetized with urethane (1 g/kg, i.p.) and their whole stomachs were collected. The mice were sacrificed by exsanguination. For determination of the TH-positive cell number in SN immunohistochemistry, the mice were transcardially perfused with 50 ml of heparinized saline, followed by 50 ml of 4% formaldehyde. Then, brain samples were isolated immediately.

Behavioral Tests. The locomotor activities of the experimental animals were measured using an open-field activity monitoring system (MAY 9908 model Activity Monitoring System: Commat Ltd, Ankara, Turkey) seven days after the injection of MPTP (DEKUNDY et al., 2006; KHALDY et al., 2003; WILLIS and ROBERTSON, 2004). The locations, total locomotor activities and the total distance of movement of the animals were calculated. For each subject 5 minutes of recording was performed (KAYIR and UZBAY, 2004). The degree of bradykinesia was measured by the pole test (KOBAYASHI et al., 1997). The pole test is used to determine movement disorders related to the basal ganglia. Seven days after treatment with MPTP, each mouse was placed head upward at the top of a rough-surfaced pole (8 mm in diameter and 50 cm in height). The mice were allowed to travel to the pole freely and move down to the floor (pre-trial). After the mice had become accustomed to the area in two/three pre-trials, the time required for the animal to turn down and descent to the floor was measured (T-total). For measurement of the neurological deficits in the groups, a fixed-speed rotarod was used (DUNHAM and MIYA, 1957). Briefly, the animals were pre-trained on an automated 4-lane rotarod unit (Ugo Basile, Italy, 5 cm diameter drums). After becoming accustomed, the mice were placed on the rod and tested for 300 s at different speeds (5, 10, 20, 30 and 40 rpm). Each animal was tested twice at each speed with a rest of 5 min between each trial.

Determination of Tyrosine Hydroxylase Immunoreactivity in Substantia Nigra. In order to determine TH immunoreactivity in the SN, brain

samples were fixed in 10% formalin (100 ml 37% formaldehyde, 900 ml distilled water, pH~7) at room temperature for 24 h. The formalin was removed by washing several times in tap water. This was followed by dehydration by immersion in 70%, 80%, 90% ethanol for 24 h each and 100% ethanol for 4 h. After dehydration, the tissues were cleared in xylene and embedded in paraffin wax. Brain samples submerged in paraffin were cut serially into 5 μm pieces from − 2.5 to − 3.6 mm in relation to the bregma. Following the process of deparaffinization, the slides were boiled in citrate buffer (pH 6.0) for 10 min to retrieve the antigens. The paraffin sections were blocked with methanol (Merck) containing 3% H_2O_2 (Merck) for 15 min after being deparaffinized. Then, following incubation with a universal blocking reagent (BioGenex) for 7 min at room temperature, the sections were incubated with mouse anti-TH primary antibody (1/1000; Abcam Inc., #ab152) for 2 h at room temperature. After the washing steps in PBS, the sections were incubated with biotinylated horse anti-mouse IgG secondary antibody (1/400; Vector Lab; #BA9200) for 1 h. Then, the sections were overlaid with LSAB streptavidin-peroxidase complex (Dako) for 20 min followed by incubation with diaminobenzidine (DAB) chromogen (Sigma-4168) for visualization of the antibody-antigen complexes. Finally, sections were counterstained with Mayer's hematoxylin (Dako) and examined with an Axioplan microscope (Zeiss, Oberkochen, Germany). The images were taken using a 5MP Canon A95 camera integrated into the microscope. The evaluation of TH immunoreactivity in the samples was performed with the image-J analysis program at 40 X magnification (NIH, Bethesda, MD, USA).

Detection of Apoptosis in Stomach. Apoptosis in all layers of the stomach wall was evaluated using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) method. Briefly, the 5 μm of thick paraffin sections of gastric tissues were deparaffinized, rehydrated and then washed twice in PBS for 5 min. After incubation with the permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) for 8 min at 4°C, the sections

were incubated with 50 μl of TUNEL reagent for 1 h at 37°C. Then, the slides were incubated with converter reagent for 30 min at 37°C. After washing, color development was performed with a Fast Red substrate solution for 10 min. Finally, TUNEL labeling was evaluated with a Cell Death Detection kit (1684809, Roche; Mannheim, Germany). The apoptotic index was determined by counting at least 100 nuclei subdivided into ten fields, chosen randomly at 40X magnification, and the apoptotic index was indicated as a percentage (%) in relation to the negative control. Negative control evaluations were performed using an enzyme-free reagent. The slides were examined with a Zeiss Axioplan Microscope, and the photographs were evaluated with Spot Advanced Software.

Determination of the Number of Mast Cells in the Stomach. Five μm thick paraffin sections containing all layers obtained from the stomach were stained with a mixture of 1% toluidine blue O (Merck-Art. 1272) and di-Natriumtetraborate (Merck-Art. 6303). Mast cells with metachromatic features were stained with purple color. The number of mast cells was determined at 40x magnification on an Axioplan microscope (Zeiss, Germany). This counting process was repeated in 6 different sections for each group and 10 photographs (40x) of each section. The average number of mast cells in 10 fields selected at random was calculated.

Measurement of TNF-α and Histamine Concentrations in the Stomach. TNF-α and histamine concentrations in gastric tissue, taken to contain all the layers, were measured with ELISA kits according to the protocols provided by the manufacturers (Cayman Chemical, #500850 and Enso Life Sciences, #ENZ-KIT140-0001, respectively). The amount of protein in the supernatant was analyzed at 595 nm with a commercial kit (Thermo Scientific, Rockford, USA) according to the method of Bradford (BRADFORD, 1976). The results were normalized by protein values and the concentrations were expressed as pg/mg protein.

Statistical Analysis. Experimental results were expressed as the mean ± the standard error of the mean (SEM). Normality distributions of the numeric variables data were analyzed by the

Shapiro-Wilk test. The differences between the groups were statistically evaluated with one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for normal distribution. Results were considered statistically significant when $P<0.05$. Statistical significance was represented as a single symbol for $P < 0.05$, a double symbol for $P < 0.01$, and a triple symbol for $P < 0.001$, as noted in the figure legends. Analysis was performed using SPSS version 13.0 software (Chicago, Illinois, USA).

Results

DHA Improved the Disturbance of Motor Functions in PD induced by MPTP. Open field test showed that the locomotor activity was significantly decreased in the Parkinson group compared with the control (Fig. 1. A, $P<0.01$). Administration of DHA significantly prevented the reduction in locomotor activity due to MPTP treatment ($P<0.001$). Rotarod performance was determined in five consecutive trials in all groups, and in-residence time on the rod at 40 rpm was evaluated. Rotarod performance was decreased due to MPTP injection compared to the control group (Fig. 1. B, $P<0.001$). DHA-pretreatment significantly prevented the reduction in rotarod performance in the Parkinson group ($P<0.001$). MPTP-induced bradykinesia in mice was evaluated by the pole test (Fig. 1. C). DHA-pretreatment significantly reversed the MPTP-induced prolongation of total time in the pole test ($P<0.001$).

DHA Prevented the Loss of Nigral TH Positive Neurons in PD induced by MPTP. The loss of dopaminergic neuron was evaluated by TH immunostaining (Fig. 2.). TH-positive neurons in SN in the control and DHA groups were similar. However, TH staining of cell bodies in SN was significantly reduced with MPTP-injection ($P<0.05$). DHA-pretreatment significantly increased the number of nigral TH positive neurons when compared to the Parkinson group ($P<0.05$).
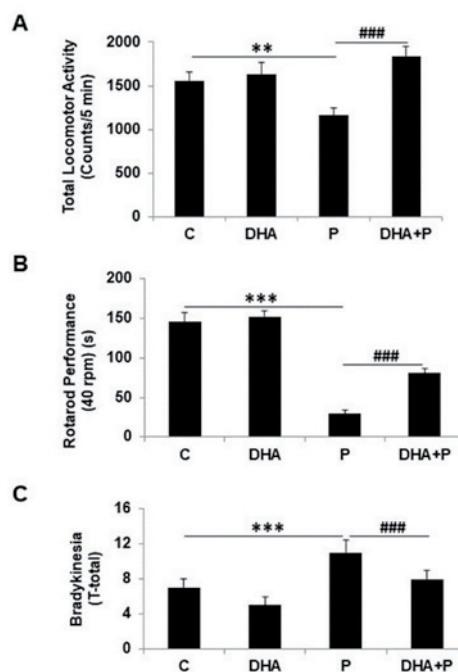

Fig. 1. The effect of DHA on motor behavioral deficits in PD induced by MPTP. A Total Locomotor Activity (total distance), ** $P<0.01$ vs the control group and ### $P<0.001$ vs the Parkinson group. B rota-rod test at 40 rpm, *** $P<0.001$ vs the control group and ### $P<0.001$ vs the Parkinson group C Bradykinesia (T-total), *** $P<0.001$ vs the control group and ### $P<0.001$ vs the Parkinson group. Data are presented as mean ± SEM.
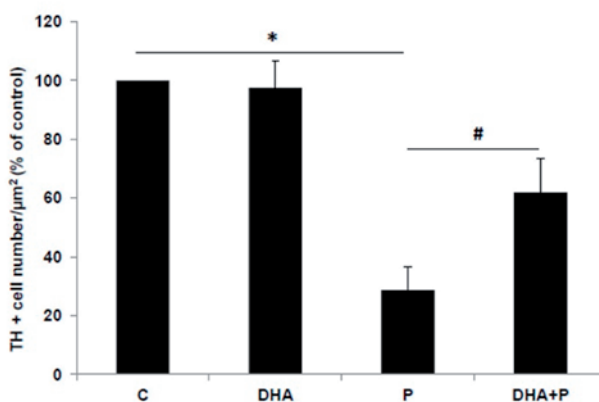

Fig. 2. The effect of DHA on MPTP-induced dopaminergic neuron degeneration in SNpc (per μm2). TH-positive neurons in coronal sections were counted at 40x magnification. Data are presented as mean ± SEM. * $P<0.05$ vs the control group and # $P<0.05$ vs the Parkinson group.

DHA Prevented the Apoptosis in the Stomach in PD induced by MPTP. After the MPTP injection, the apoptotic changes were observed mostly in the connective tissue in the stomach (Fig. 3. A). Contrary to our expectation, ENS seemed unaffected by the apoptotic process, because there was no apoptosis in the myenteric and submucosal plexus. Furthermore, when DHA was administered alone, it did not affect the apoptosis compared with the control group (Fig. 3. B). It was found that the number of apoptotic cells (as a percentage) increased significantly in the Parkinson group compared with control group (P<0.001). However, DHA reduced MPTP-induced apoptotic changes in the stomach (P<0.05).

DHA Reduced the Concentration of TNF-α in the Stomach in PD induced by MPTP. The level of TNF-α in control group was 442 ± 32 pg/mg protein (Fig. 4), but the TNF-α concentration significantly increased to 886 ± 64 pg/mg protein in the MPTP-treated group (P<0.001). However, pre-treatment with DHA prevented the increase in the TNF-α concentration in the MPTP-injected group (603 ± 55 pg/mg protein, P<0.01).
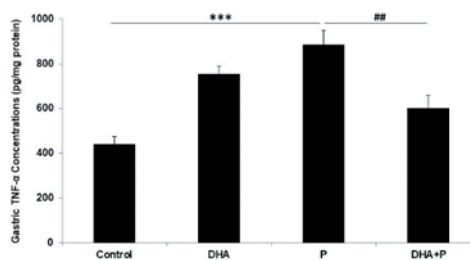
Fig. 4. The effect of DHA on TNF-α concentration in gastric tissue in PD induced by MPTP. Data are presented as mean ± SEM. ** P<0.001 vs the control group and # P<0.01 vs the Parkinson group.

DHA reduced the Number of Mast Cells and Histamine Concentration in the Stomach in PD induced by MPTP. As seen in Figs. 5A and B, the number of mast cells in the stomachs of the Parkinson group greatly increased compared with the control group (P<0.001). DHA administration prevented the increase in the number of gastric mast cells in MPTP-induced PD (P<0.05).
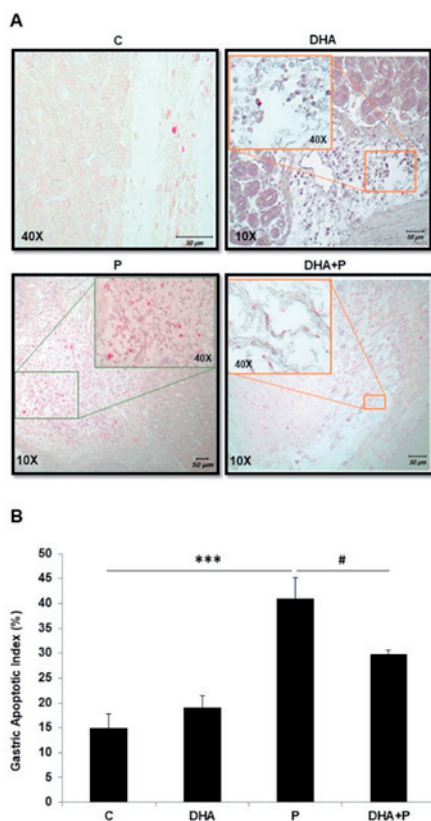

Fig. 3. The effect of DHA on apoptosis in gastric tissue in PD induced by MPTP. **(A)** The representative TUNEL staining images in gastric tissues of experimental groups (magnifications 10x and 40x) **(**Scale bar, 50 μm). **(B)** The graphic of the mathematical values of the apoptotic index (%) in gastric samples. Data are presented as mean ± SEM. *** P<0.001 vs control group and # P<0.05 vs the Parkinson group.
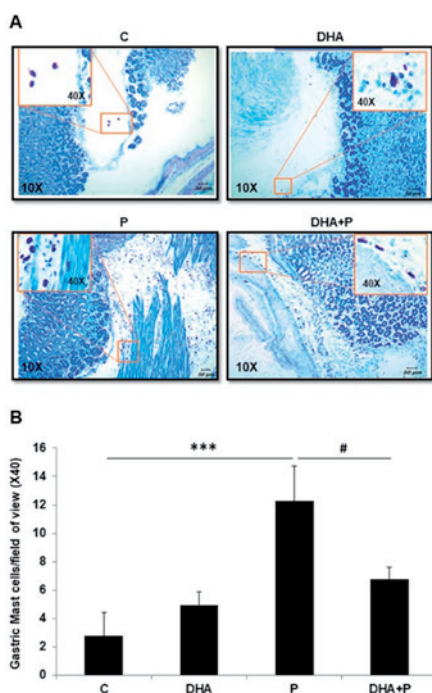
Fig. 5. The effect of DHA on the mast infiltration in gastric tissue in PD induced by MPTP. **(A)** The representative immunohistochemically staining of the mast cell infiltration with toluidine blue staining in the experimental groups (magnifications 10x and 40x) (Scale bar, 50 μm). **(B)** The graph of the mathematical values of mast cell count in gastric tissue (per μm^2). Data are presented as mean ± SEM. *** $P<0.001$ vs the control group and # $P<0.05$ vs the Parkinson group.

Similar to the change observed in the number of mast cells, histamine concentration increased markedly with MPTP administration (152±17 pg/mg protein, Fig. 6) compared with the control group (52±9 pg/mg protein, $P<0.001$). DHA administration prevented the increase due to MPTP-induced PD in histamine concentration in the gastric tissue (80±8 pg/mg protein, $P<0.01$).
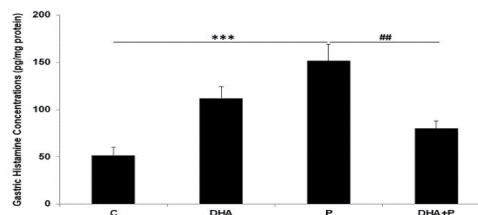

Fig. 6. The effect of DHA on histamine concentration in gastric tissue in PD induced by MPTP. Data are presented as mean ± SEM. ** $P<0.001$ vs the control group and # $P<0.01$ vs the Parkinson group.

Discussion

Locomotor activity, motor coordination and balance are known to be impaired in PD induced by MPTP in mice. In this study, motor coordination and balance were evaluated by the rotarod test (ROZAS et al., 1998). Similar to previous studies, we observed that the riding time at 40 rpm and total locomotor activity were decreased in the Parkinson group compared to the control (GAMBHIR et al., 2011; GOES et al., 2014). In the DHA+P group, the riding time and motor functions were increased compared to the Parkinson group. The pole test is used to determine movement disorders which are related to basal ganglia. The findings obtained from the pole test showed the return and landing time was prolonged in the Parkinson group and shorter in the DHA-P group. The improving effect of DHA on dopaminergic neurons in the SN and motor coordination was shown in this study, as previously (OZKAN et al., 2016; OZSOY et al., 2011). DHA prevents the loss of neurons that are important in motor activities due to its anti-inflammatory and anti-apoptotic effects. Furthermore it is known that DHA enhances striatal dopamine synthesis in a Parkinson's model induced by 6-OHDA in rats (CHITRE et al., 2020). Systemically administered, MPTP has lipophilic properties, it can cross the blood-brain barrier, and is converted by monoamine oxidase-B into 1-methyl-4-phenylpyridinium that inhibits the mitochondrial complex I activity in the dopaminergic neurons of the SN, inducing dopaminergic neurodegeneration. To show the

successful implementation of the Parkinson's model, in this study the TH-positive cell count was determined immunohistochemically in the SN (GOETZ, 2011). It was found that MPTP injection reduced the number of TH-positive neurons by 75% compared to the control group. In the DHA+P group, the TH-positive neuron count was double compared to the P group; however, it did not reach the level of the control. No difference was observed between the DHA and the control group. Similar to our results, previous studies reported that the administration of DHA prevented the MPTP-induced decrease in the number of TH-positive neurons in the SN (OZKAN et al., 2016; OZSOY et al., 2011).

Apoptosis is defined as the self-destruction of cells by the activation of death receptors and mitochondrial pathways. We observed that there was no evidence related to apoptosis in the enteric plexus neurons. We did not investigate α-synuclein occlusions in the present study, but previous studies showed that, in addition to constipation, there was dopaminergic neuron loss within both the myenteric and submucosal plexus, and α-synuclein aggregation in the mice intestine in PD induced by MPTP, but not in the stomach (NATALE et al., 2010). Nevertheless, we observed widespread apoptosis in other structures in the stomach wall, especially in the connective tissue. Current information related to this subject suggests that MPTP, to which dopaminergic neurons are especially sensitive, may have affected the nigral neurons more than the enteric neurons in the stomach. At the same time, the pathology in the SN may have affected the gastric tissue in this experimental model. Interestingly, according to our study in addition to apoptosis, increased numbers of mast cells were found in the connective tissue. In parallel with the increase in the mast cell count, there was also an increase in histamine levels in the stomach. The administration of DHA inhibited the apoptotic alterations in the stomach due to PD induced by MPTP. In addition, the number of mast cells and the histamine concentration in the stomach were decreased with DHA-pretreatment. These results suggest that pretreatment with DHA by gavage may have protected the dopaminergic

neurons in the SN and the stomach against the effects of MPTP.

Gastroparesis, nausea, ulcer, vomiting, early satiety, and bloating are common manifestations of PD. The disorders in GI function in different PD models have been the subject of many studies (GREENE, 2011; WAKABAYASHI et al., 2007). The exact cause of GI dysfunction in PD is still not clearly known, but, these symptoms are believed to be associated with bilateral brain-gut communication and inflammation (GREENE, 2011). It is known that peripheral inflammation may enhance the sensitivity of dopaminergic neurons to degeneration (GARRIDO-GIL et al., 2018). In this context, some clinical studies suggest that the prevalence of idiopathic PD is lower in chronic non-steroidal anti-inflammatory drug users (TON et al., 2006).

During the examination of the histological samples, an interesting finding attracted our attention. Although there was apoptosis in the connective tissue elements, we observed an increase in the number of mast cells in the mucosa. We therefore measured the amount of histamine in the stomach tissue samples and found an increase due to PD. We also measured the amount of TNF-α, a pro-inflammatory cytokine that induces apoptosis in stomach tissue. The increased TNF-α concentration in the stomach is accompanied by an increase in the number of mast cells, suggesting that TNF-α may be released by the mast cells in PD induced by MPTP. This suggestion is consistent with studies showing that mast cells can secrete proinflammatory cytokines, including TNF-α (KEMPURAJ et al., 2017). Mast cells have a role in normal conditions in tissue repair and host defense, and pathological conditions, such as autoimmunity, allergy and inflammation (VOEHRINGER, 2013). In previous studies, activated mast cells were shown to cause neuroinflammation by secretion of histamine, serotonin, kinins, leukotrienes, cytokines and chemokines, and proteolytic enzymes in the brain (KEMPURAJ et al., 2017). Although mast cells are associated with the functions of the endothelial and epithelial cells and neurons in the GI tract, the relationship between mast cells and GI dysfunction in PD pathogenesis is not yet known. Mast cells

may have a role in the modulation of inflammation and brain-gut communication. Our data showed that DHA reduced the expression of TNF-α and mast cell count in the stomach of mice with Parkinson's. Therefore, the reducing effect of DHA on apoptosis may depend on the level of TNF-α and mast cell activation. It was shown that the Fas antigen is a cell surface receptor that triggers apoptosis in cells when bound to the Fas ligand such as TNF-α. DHA decreases TNF-α production in LPS-stimulated macrophages when compared to the control cells (WELDON et al., 2007).

Conclusions

In conclusion, increased apoptosis and also mast cell count were observed in the stomach in mice with PD induced by MPTP. These changes may be related to GI dysfunction in PD. DHA-pretreatment prevented apoptosis and the increase in the mast cell count in mouse stomachs related to MPTP. It is not possible to determine from the findings obtained from this study whether the pathological changes in the stomach cause PD or PD causes the pathological changes in the stomach. Further studies are needed to understand whether pathological changes in the stomach are the cause or result of PD. However, DHA has a protective effect against changes in the stomach in PD induced by MPTP in mice. This result may provide a treatment approach to prevent GI dysfunction observed in PD.

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SAŽETAK

U radu je istražen zaštitni učinak dokozaheksaenske kiseline (DHA) na želučane promjene u muških miševa s Parkinsonovom bolešću (PD), izazvanom 1-metil-4-fenil-1.2.3.6.-tetrahidropiridinom (MPTP). C57BL/6 miševi su podijeljeni u četiri skupine (n = 10 po skupini): kontrolnu skupinu, skupinu DHA, skupinu Parkinson i skupinu DHA + Parkinson. DHA je primjenjivana pomoću sonde 30 dana. Dvadeset treći dan životinjama u skupinama Parkinson i DHA + Parkinson intraperitonealno je injiciran MPTP. Sedam dana poslije primjene MPTP-a izmjerena je lokomotorna aktivnost, bradikinezija i proveden rotarod-test. Procijenjeni su ekspresija tirozin-hidroksilaze (TH u substantia nigra), apoptotički indeks, koncentracije faktora tumorske nekroze alfa (TNF-α) i histamina te broj mastocita u želucu. Primjena DHA znakovito je prevenirala smanjenje motoričkih funkcija (P < 0,001) i TH neurona (P < 0,05) te apoptoze (P < 0,05), a povećala koncentraciju TNF-α (P < 0,01) u želucu. Porast broja mastocita u želučanoj stijenci utvrđen je u skupinama s PD (P < 0,001). DHA je prevenirala porast broja mastocita (P < 0,05) i razine histamina (P < 0,01) zahvaljujući PD-u. Zaključeno je da primjena MPTP-a u miševa uzrokuje promjene u želucu i motoričkim funkcijama te da DHA utječe na smanjenje tih promjena.

Ključne riječi: dokozaheksaenska kiselina; želučano tkivo; MPTP; apoptoza; mastociti; miševi