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The Effects of Vasointestinal Peptide and Naringenin on Rotenone-Induced Experimental Model of Parkinson's Disease

Vazointestinal Peptid ve Naringeninin Rotenon Kaynaklı Deneysel Parkinson Hastalığı Modeli Üzerine Etkileri

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ABSTRACT

Aim: The aim of this study was to evaluate the intraperitoneal administration of naringenin and vasointestinal peptide (VIP), which are shown effective in various scientific studies, in terms of anti-Parkinsonian activity in rats.
Material and Methods: Forty-eight Wistar albino female rats were divided into 4 groups. No intervention was made in the control group, rotenone was given to the RT group, rotenone and VIP (25 ng/kg) to the RT+VIP group, and rotenone and naringenin (10 mg/kg) to the RT+NG group. All treatments were administered intraperitoneally for 14 days. The hole and board method was used to show the effects of the Parkinson's model on behavior. On the last day of the experiment, motor tests were carried out with the hole and board apparatus. After the study was completed, biochemical analyzes were performed from brain tissue samples.
Results: In comparison to the RT group, while the alpha-sync level in the RT+NG (p=0.023), malondialdehyde (MDA) levels both in the RT+VIP (p=0.039) and RT+NG (p=0.032), and superoxide dismutase (SOD) inhibition in the RT+VIP (p=0.042) groups decreased significantly, the 8-OHdG levels in the RT+VIP (p=0.042) and RT+NG (p=0.034) groups increased significantly. Statistically significant improvement was found both in biochemical and motor activities with the VIP and naringenin treatments applied.
Conclusion: According to the results obtained, the symptoms of Parkinson's disease were formed biochemically by rotenone application. The administration of VIP and naringenin treatments has shown positive effects experimentally and has been promising as an adjunct treatment element in the fight against Parkinson's disease.
Keywords: Parkinson's disease; rats; rotenone; vasointestinal peptide.

ÖZ

Amaç: Bu çalışmanın amacı, çeşitli bilimsel çalışmalar ile etkili oldukları gösterilmiş olan naringenin ve vazointestinal peptidin (VIP) intraperitoneal olarak uygulanmasının ratlarda anti-Parkinson aktivitesi açısından değerlendirilmesidir.
Gereç ve Yöntemler: Kırk sekiz adet Wistar albino dişi rat 4 gruba ayrıldı. Kontrol grubuna herhangi bir müdahale yapılmadı, RT grubuna rotenon verilirken, RT+VIP grubuna rotenon ve VIP (25 ng/kg) ve RT+NG grubuna ise rotenone ve naringenin (10 mg/kg) verildi. Tüm tedaviler 14 gün süreyle intraperitoneal yolla uygulandı. Parkinson modelinin davranış üzerindeki etkilerini göstermek için hole and board yöntemi kullanıldı. Deneyin son günü hole and board aparatı ile motor testleri yapıldı. Çalışma tamamlandıktan sonra alınan beyin dokusu örneklerinden biyokimyasal analizler yapıldı.
Bulgular: RT grubuyla karşılaştırıldığında, RT+NG (p=0,023) grubunda alfa senkronizasyon düzeyi, hem RT+VIP (p=0,039) hem de RT+NG (p=0,032) gruplarında malondialdehit (MDA) düzeyleri ve RT+VIP (p=0,042) grubunda süperoksit dismutaz (SOD) inhibisyonu anlamlı olarak azalırken, RT+VIP (p=0,042) ve RT+NG (p=0,034) gruplarında ise 8-OHdG seviyeleri anlamlı şekilde arttı. Uygulanan VIP ve naringenin tedavileri ile hem biyokimyasal ve hem de motor aktivitelerinde istatistiksel olarak anlamlı şekilde düzelme saptandı.
Sonuç: Elde edilen sonuçlara göre rotenon uygulaması ile Parkinson hastalığının semptomları biyokimyasal olarak oluşturulmuştur. VIP ve naringenin tedavilerinin uygulanması deneysel olarak olumlu etkiler göstermiştir ve Parkinson hastalığı ile mücadelede yardımcı bir tedavi unsuru olarak umut verici olmuştur.
Anahtar kelimeler: Parkinson hastalığı; rotenon; sıçanlar; vazointestinal peptit.

INTRODUCTION

Parkinson's disease is a multisystem progressive disease characterized by premotor and motor symptoms clinically due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) and denervation of dopaminergic pathways, in which intracytoplasmic inclusions are seen in the neurons affected in the central nervous system (1,2). A long period characterized by premotor symptoms, usually the appearance of motor symptoms, is referred to as the 'preclinical period' (3). When the disease is diagnosed, in other words, when clinically motor symptoms are observed, 50-70% of dopaminergic neurons in the substantia nigra pars compacta have degenerated. The reason is, it has been reported that it may be too late for neuroprotective applications that may slow down neurodegeneration or have an effect (4,5). Many experimental models have been developed to understand Parkinson's disease (3). One of the most widely used models is the rotenone model (6). Rotenone is a toxic substance used in pest control, a member of the rotenoid family, which is one of the natural cytotoxic compounds obtained from the extracts of tropical plants. It is lipophilic and can easily reach organs through circulation. Rotenone binds to the same site as MPP+ and inhibits mitochondrial complex I. Intravenous exposure to low-dose rotenone causes selective degeneration of nigrostriatal dopaminergic neurons with the formation of α-synuclein positive LC-like inclusions in rats. It has been reported that rotenone, which is lipophilic, easily crosses the blood-brain barrier. Rotenone accumulating in mitochondria blocks the complex-I unit of the electron transport chain (2,3).

Many of the features found in Parkinson's pathology include reactive oxygen species production, systemic mitochondrial degradation, microglial activation, α-synuclein phosphorylation, aggregation, and Lewy pathology, selective nigrostriatal dopaminergic degeneration, ubiquitin-proteasomal dysfunction, and L-3,4-dihydroxyphenylalanine (levodopa; L- many symptoms such as DOPA) responsive motor deficits, depletion of tyrosine hydroxylase immunoreactivity, oxidative damage are observed after rotenone administration (7-9). Mitochondrial complex I inhibition and reactive oxygen species production are key mechanisms for the degeneration of dopaminergic neurons. Increased intracellular oxidative stress also causes dysfunction of the ubiquitin proteasomal system, which provides degradation of misfolded protein aggregates (10). As a result, mitochondrial membrane potential decreases, intracellular calcium homeostasis is disturbed and mitochondria are destroyed (mitophagy), neurodegeneration is observed (11).

Vasointestinal peptide (VIP) is a peptide consisting of 28 amino acids, structurally belonging to the family of gastrointestinal tract peptide hormones such as glucagon, secretin, gastric inhibitory peptide (GIP), and growth hormone-releasing hormone (GHRH) (12,13). VIP has a wide range of biological activities and participates in the regulation of a wide range of physiological functions such as circulatory, respiratory, gastrointestinal, endocrine, and immune systems (12,14). VIP is the main neuropeptide in the brain with its neurotransmitter, neuromodulator, neurotrophic, anti-inflammatory, antioxidant, and antiapoptotic properties (15), it stimulates astrocyte mitosis and neuronal growth in the CNS (16), it provides neuronal vitality and prevents cell death against glutamate excitotoxicity (17) were reported. VIP is a molecule with tissue and cell protective properties. It has been shown in various studies to protect tissues against the undesirable damage of septic shock, Chron's disease, hemorrhagic shock, ischemia-reperfusion, and rheumatoid arthritis, and increase neuronal survival (14,18). It is suggested that VIP plays an important role in the protection and development of neurons in traumatic situations in the brain (19).

Naringenin is a flavonoid from a subclass of flavanones, which is found in various citrus fruits such as tomatoes and bergamot and can also be found in glycoside form (20). Naringenin, chemically named 2,3-dihido-5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, indicates a molecular weight of 272.26 ($C_{15}H_{12}O$). This molecule is insoluble in water and dissolves in organic solvents such as alcohol (21). Flavonoids are phenolic compounds commonly found in fruits and vegetables that exhibit strong antioxidant activity and reduce the formation of free radicals (22). Naringenin exhibits antiatherogenic and anti-inflammatory effects, supporting carbohydrate metabolism, increasing antioxidant defense, scavenging reactive oxygen species, and modulating immune system activity, including reduction in lipid peroxidation biomarkers and protein carbonylation (23). It also promotes the oxidation of fatty acids and effectively disrupts the accumulation of plasma lipids and lipoproteins, which impairs the accumulation of lipids in the liver and prevents fatty liver. It has been reported to contribute significantly to modulating signaling pathways related to fatty acid metabolism (24).

This study aimed to investigate the neuroprotective effects of intraperitoneal use of VIP and naringenin agents in the experimental Parkinson's model created by rotenone administration.

MATERIAL AND METHODS

In this experimental study and the experimental interventions it contains, it has been approved that there is no ethical objection to the decision of Aydın Adnan Menderes University Animal Experiments Local Ethics Committee with the decision numbered 054, dated 21.05.2019. In the study, 48 Wistar albino male rats with an average weight of 300-350 grams, 12 weeks old, found in the Experimental Animals Laboratory of Aydın Adnan Menderes University were used. All rats were kept in rooms with 12 hours of dark and 12 hours of light circadian rhythm, 22±1°C temperature, and 40-60% relative humidity throughout the experiment. During the experiment, rats were fed with standard pellet feed ad libitum, and free-access city water was used as drinking water.

Rotenone (Sigma Aldrich) to be administered to rats was prepared by dissolving in dimethylsulfoxide (DMSO) (Sasol). It was administered intraperitoneally with an insulin injector at 1.25 mL/kg per rat. In the first 30 minutes after the application, some physical changes were observed in some of the rats. Mortality occurred when the rats remained in an inactive lying position after postural disorder and decreased breathing. When the second 45th minute was entered, this and similar situations were encountered in other groups as well, and the number of rats

starting with n=48 in total was n=36. The VIP, which was prepared in saline starting 1 hour after the Parkinson's model was created, was administered ip at 25 ng/kg, was applied as VIP treatment, and was continued every other day for 14 days (25). Naringenin was administered as 10 mg/kg in pure form on a repetitive day after the rotenone application (26). Experimental animals were randomly selected, 12 rats were in each group, and 4 groups were formed. The groups were; the Rotenone group (RT), the Rotenone+Vasointestinal Peptide Group (RT+VIP), and the Rotenone+Naringenin Group (RT+NG). Alpha-sync, 8-OHdG, malondialdehyde (MDA), and superoxide dismutase (SOD) kits were purchased to perform biochemical tests, and measurements were performed following the kit procedure.

Hole and Board Test

At the end of the 14th day, the Hole and board test setup was used to perform the motor analysis on the last day of the experiment. This arrangement is structured as follows: Perforated wooden apparatus, 68 cm x 68 cm, consisting of a wooden gray box, 40 cm high, with the front side open and the other three closed, and the hole diameter (may vary depending on the size of the experimental animal) is 9 cm, The box with 16 holes was raised 28 cm from the ground on a wooden stand (Figure 1). To ensure that the experimental animal is accustomed to the environment, it was put into the apparatus 3 times at different times. After the acclimatization period was completed, 5 minutes of shooting was taken for each rat with a fixed video camera that could fully see the experimental setup. Experimental animals performed hovering and exploratory movements on the apparatus. Parameters used in the Hole and board test; (i) Visit to the center: the animal moves from one area of the open area to another (all four claws must be placed on the floor of a new area), (ii) Head dipping: the animal places its head in one of the holes to a minimum depth with its ears flush with the floor of the device (the animal a new head tilt is recorded when he raises his head) a full movement is achieved when he pulls it out of the hole before continuing), (iii) Raising: the animal is fixed on its back paws and lifts its front paws off the ground, extending its body vertically, (iv) Number of holes: the animal extends and retracts its head without dipping its head into one of the holes.

Statistical Analysis

All statistical analyses were performed via IBM SPSS Statistics 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The distributions of measurements were evaluated by Shapiro-Wilk's test and skewness/kurtosis statistics. The homogeneity of the variances among groups was examined by Levene's test. Data were provided by mean±standard deviation. Groups were compared by ANOVA. Tukey's HSD test was performed, if necessary. A p-value <0.05 was accepted as statistically significant.

RESULTS

Biochemical Analysis Findings

In the results of the biochemical analysis performed on the samples taken from the striatum region, alpha-sync levels were found to be significantly lower in the control group than in the RT group (p=0.008). While no significant difference was found in the RT+VIP group (p=0.085), there was a significant decrease in the alpha-sync levels of the RT+NG group compared to the RT group (p=0.023).

8-OHdG levels in the striatum decreased significantly in the RT group compared to the control group (p=0.009) and increased closer to the control group in the RT+VIP and RT+NG groups. It was determined that 8-OHdG levels in the RT+VIP (p=0.042) and RT+NG (p=0.034) groups increased significantly compared to the RT group.

The MDA level in the RT group increased significantly compared to the control group (p=0.021), whereas it decreased significantly in the RT+VIP (p=0.039) and RT+NG (p=0.032) treatment groups.

While the SOD inhibition in the RT group increased significantly (p=0.038) compared to the control group, it was significantly lower in the RT+VIP (p=0.042) group compared to the RT group. The lowest inhibition was in the control group (Table 1).

Motor Test Findings

While the most head dipping movement was detected in the control group, this movement was significantly less in the RT group compared to the control group (p=0.007). Although there was a slight increase in the treatment groups, it was not statistically significant.

According to the number of holes visited, it was found that the group with the highest number of visits was the control group, and the lowest number was the RT group (p=0.019). There was an increase in both treatment groups compared to the RT group and the increase in the RT+VIP group was significant (p=0.031), while was not in the RT+NG group. The highest number of visits to the center was in the control group and the lowest was in the RT group (p=0.036). Although this number increased in the RT+VIP group, no significant difference was found (p=0.543). Also, there was no significant difference between the RT and RT+NG groups (p=0.961).

The highest number of rearing movements was found in the control group and the lowest in the RT group (p=0.037). Although this number increased in the RT+VIP (p=0.720) and RT+NG (p=0.994) treatment groups, no significant difference was found when compared to the RT group.

DISCUSSION

Creating an accessible model for Parkinson's disease studies is challenging. In the studies conducted by Sonia Angeline et al. (27) and Lapointe et al. (28), it was determined that the rotenone-induced Parkinson-like disease model causes neuronal damage in the important nigra and striatum, and leads to behavioral changes such as motor

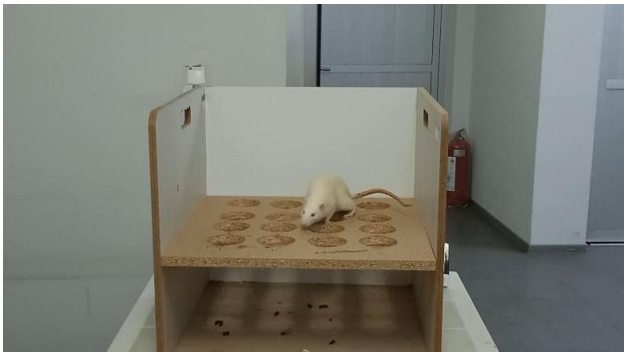

Figure 1. Hole and board assembly

Table 1. Biochemistry analysis results

	Control	RT	RT+VIP	RT+NG
Alfa-sync (pg/gr)	16.823±2.420	29.593±6.112	22.061±4.001	19.204±3.443
8-OHdG (µg/gr)	43.273±16.148	37.701±13.988	41.377±10.046	39.771±12.089
MDA (mU/ml)	1.392±0.305	1.952±0.361	1.584±0.034	1.510±0.694
SOD inhibition (%)	0.491±0.298	0.621±0.068	0.512±0.068	0.565±0.296

RT: rotenone, RT+VIP: rotenone+vasointestinal peptide, RT+NG: rotenone+naringenin, MDA: malondialdehyde, SOD: superoxide dismutase, Tukey HSD post hoc test results; Alfa-sync: RT vs Control: p=0.008, RT vs RT+VIP: p=0.085, RT vs RT+NG: p=0.023; 8-OHdG: RT vs Control: p=0.009; RT vs RT+VIP: p=0.042; RT vs RT+NG: p=0.034; MDA: RT vs Control: p=0.021; RT vs RT+VIP: p=0.039; RT vs RT+NG: p=0.032; SOD inhibition: RT vs Control: p=0.038; RT vs RT+VIP: p=0.042; RT vs RT+NG: p=0.055

Table 2. Motor test results

	Control	RT	RT+VIP	RT+NG
Head dip (score)	9.634±2.723	4.222±1.725	5.384±1.300	4.305±1.704
Hole visit (score)	8.250±3.451	4.558±1.591	7.126±1.134	5.200±1.140
Center visit (score)	2.000±0.816	1.250±0.500	1.500±0.554	1.200±0.425
Rampancy (score)	3.631±1.688	2.000±1.000	2.384±1.194	2.000±1.700

RT: rotenone, RT+VIP: rotenone+vasointestinal peptide, RT+NG: rotenone+naringenin, Tukey HSD post hoc test results; Head dip: RT vs Control: p=0.007; RT vs RT+VIP: p=0.087; RT vs RT+NG: p=0.063; Hole visit: RT vs Control: p=0.019; RT vs RT+VIP: p=0.031; RT vs RT+NG: p=0.074; Center visit: RT vs Control: p=0.036; RT vs RT+VIP: p=0.543; RT vs RT+NG: p=0.961; Rampancy: RT vs Control: p=0.037; RT vs RT+VIP: p=0.720; RT vs RT+NG: p=0.994

skill disorders and biochemical disorders. In our study, it was observed that the biochemistry and motor behavior results of the rotenone-applied group were impaired compared to the control group.

Experimental studies in this area reveal the promising role of flavonoids (29). It has been shown to improve cognitive function, reduce motor complications, and protect biochemically by protecting sensitive neurons (30). The study by Datla et al. (31) showed that these promising properties of flavonoids can be used as potent neuroprotectors for naringenin. The results we obtained in our study also showed that the application of naringenin caused improvements in both biochemical and motor activity results.

It was observed that the inhibition level of MDA and SOD decreased significantly after the treatment of rotenone-treated rats with VIP. While describing the neuroprotective effect of VIP, it is assumed that it increases astrocyte mitosis and stimulates the release of astrocyte-derived neurotrophic molecules such as ADNP (16,32). In their in vivo study by giving VIP antagonists in mice, it was shown that there was a dramatic loss of astrocyte in the neocortex, and neocortical astrogenesis occurred with VIP administration and this loss was reversed (33). The results of our study support these findings.

In the study conducted by Liu et al. (34), it was determined that the results obtained in the board and hole tests applied to rats with the Parkinson's model deteriorated significantly compared to the results in the control group. It was observed that rats with Parkinson's-like features had decreased ability to pass through, the number of rearing, and the number of holes visited. Wang et al. (35), on the other hand, applied behavioral tests on rats with Parkinson's-like effects in their experimental Parkinson's study and found a significant difference from the control group as a result of hole and board tests. In the study of Saleem et al. (36), the experimental results of Prunus armeniaca L. extract were investigated in rats with Parkinson's-like effects, and they determined that both biochemistry and motor activity results approached the control group. The results obtained in our study also support the findings obtained in these studies. Naringenin and VIP applications reduced the negative effects of the rotenone application.

In addition to nigrostriatal degeneration, when rotenone is administered by intraperitoneal injection, it causes behavioral deficits responsive to the dopamine agonist apomorphine, suggesting that these observed deficits are specific to dopamine loss. With these features, it has been determined that symptoms similar to Parkinson's disease in humans occur (6). Although it is known that the intrinsic pathway, that is, the mitochondrial pathway, is of great importance in models that occur with mitochondrial complex-1 inhibition, it is reported that the extrinsic pathway may be effective on this intrinsic pathway (1). The primary goal in the treatment of Parkinson's disease is to improve the patient's quality of life by treating motor and non-motor findings (37). The fact that only palliative treatment is possible with drugs aimed at increasing dopaminergic neurotransmission makes studies on potential drug candidates with neuroprotective effects in Parkinson's disease inevitable (3). The results revealed in our study showed that both naringenin and VIP have positive effects biochemically. When motor activity test findings are added to these results, it is thought that naringenin and VIP application will be effective elements in the Parkinson's treatment process in the future.

CONCLUSION

As a result, the results show that rotenone application negatively affects biochemical results and motor behaviors, and findings similar to Parkinson's disease are obtained. It was shown that the applied VIP and naringenin improved the negative results and changed the biochemical and motor test results similarly to the control group. The results show that VIP and naringenin, which did not cause any harm in the administration, are agents that can be added to the treatment of Parkinson's disease. Further molecular studies will reveal the pathways through which these two therapeutic agents act.

Ethics Committee Approval: The study was approved by the Animal Experiments Local Ethics Committee of Aydın Adnan Menderes University (21.05.2019, 054).

Conflict of Interest: None declared by the authors.

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