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Investigation of the Effect of Phosphodiesterase Type 9 Inhibitor PF-04447943 on Spatial Memory in Mice Using the Radial Arm Maze Test

Fosfodiesteraz Tip 9 İnhibitörü PF-04447943'ün Farelerde Uzamsal Bellek Üzerindeki Etkisinin Işınsal Kollu Labirent Testi Kullanılarak Araştırılması

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Abstract

Objective: Phosphodiesterase-9 (PDE9) inhibitors have attracted interest for improving cognitive function by increasing intracellular cGMP, an important mediator of learning and memory. The nitric oxide (NO)/cGMP pathway plays a key role in synaptic plasticity and hippocampal long-term potentiation (LTP), while inhibition of neuronal nitric oxide synthase (nNOS) impairs cognition. This study evaluated the effects of the PDE9 inhibitor PF-04447943 on spatial memory in mice and its interaction with nNOS inhibition induced by 7-nitroindazole (7-NI).

Materials and Methods: Fifty male BALB/c mice (7–8 weeks old, 20–30 g) were randomly divided into five groups (n = 10 per group): control, PF-04447943 (1 and 3 mg/kg), 7-Nitroindazole (7-NI; 5 mg/kg), and a combination treatment group (5 mg/kg 7NI and 3 mg/kg PF-04447943). Drugs were administered intraperitoneally (i.p.). The radial arm maze test was used to measure spatial memory by recording arm reentries (errors) and task completion time (latency).

Results: PF-04447943 (1 and 3 mg/kg, i.p.) significantly decreased the number of errors and latency at 3 mg/kg. The nNOS inhibitor 7-NI (5 mg/kg) significantly increased both values. The combined treatment group made notably fewer mistakes and had significantly reduced latency than the 7-NI group, yet their performance was not completely restored to control levels.

Conclusions: Inhibiting PDE9 may enhance spatial memory and partially alleviate cognitive impairment associated with the NO pathway; however, intact NO signalling seems essential for complete cognitive recovery.

Keywords: Mice, PF-04447943, phosphodiesterase type 9 inhibitor, radial arm maze, spatial memory

Öz

Amaç: Fosfodiesteraz-9 (PDE9) inhibitörleri, öğrenme ve belleğin önemli bir düzenleyicisi olan hücre içi cGMP düzeylerini artırarak bilişsel işlevleri iyileştirme potansiyelleri nedeniyle ilgi görmektedir. Nitrik oksit (NO)/cGMP yolu, sinaptik plastisite ve hipokampal uzun süreli potansiyasyon (LTP) süreçlerinde önemli bir rol oynarken, nöronal nitrik oksit sentazın (nNOS) inhibisyonunun bilişsel işlevleri bozduğu gösterilmiştir. Bu çalışmada, PDE9 inhibitörü PF-04447943'ün farelerde uzamsal bellek üzerindeki etkileri ve 7-nitroindazol (7-NI) ile oluşturulan nNOS inhibisyonu ile etkileşimi araştırılmıştır.

Materyal ve Metot: Elli adet erkek BALB/c fare (7–8 haftalık, 20–30 g) rastgele beş gruba ayrılmıştır (her grup n = 10): kontrol grubu, PF-04447943 (1 ve 3 mg/kg), 7-Nitroindazol (7-NI; 5 mg/kg) ve kombinasyon tedavisi grubu (5 mg/kg 7-NI ve 3 mg/kg PF-04447943). İlaçlar intraperitoneal (i.p.) yolla uygulanmıştır. Uzaysal bellek, radyal kollu labirent testi kullanılarak, kol tekrar girişleri (hata) ve görev tamamlama süresi (latans) kaydedilerek değerlendirilmiştir.

Bulgular: PF-04447943 (1 ve 3 mg/kg, i.p.) uygulaması, özellikle 3 mg/kg dozunda hata sayısını ve latensi anlamlı şekilde azalttı. nNOS inhibitörü 7-NI (5 mg/kg) ise her iki parametreyi de anlamlı olarak artırdı. Kombinasyon tedavisi uygulanan grupta, 7-NI grubuna kıyasla hata sayısı ve latens belirgin şekilde daha düşük bulundu; ancak performans tamamen kontrol düzeyine dönmedi.

Sonuç: PDE9'un inhibisyonu uzaysal hafızayı artırabilir ve NO yolakla ilişkili bilişsel bozulmayı kısmen azaltabilir; ancak tam bilişsel iyileşme için sağlam NO sinyalizasyonunun gerekli olduğu görülmektedir.

Anahtar Kelimeler: Fare, fosfodiesteraz tip 9 inhibitörü, PF-04447943, radyal kollu labirent, uzaysal bellek

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INTRODUCTION

Cognitive impairment is one of aging main symptoms and is severe in neurodegenerative diseases such as Alzheimer's disease (AD). Current AD treatments mainly act on cholinergic pathways or glutamate-mediated excitotoxicity; therefore, they only relieve disease symptoms without affecting the main disease mechanisms. Therefore, finding new molecular targets involved in synaptic plasticity and memory formation is an important research goal.[1–3]

The nitric oxide (NO)/soluble guanylyl cyclase (sGC)/cyclic guanosine monophosphate (cGMP) cascade is one of the intracellular signaling pathways that has received considerable attention in relation to learning and memory.[4,5] NO functions as a retrograde messenger within the central nervous system and is essential for long-term potentiation (LTP), a cellular mechanism associated with memory formation. When NO activates sGC, it raises the levels of cGMP inside cells. This then activates protein kinase G (PKG) and other signaling molecules that are involved in gene transcription and synaptic remodelling.[5] Problems with this pathway have been linked to problems with learning tasks that depend on the hippocampus.[6,7]

By breaking down cAMP and cGMP, phosphodiesterases (PDEs) control cyclic nucleotide signaling.[3] Of the 11 PDE families that have been found so far, PDE9 has the strongest affinity to cGMP and is highly expressed in brain areas related to cognition, such as the hippocampus and cerebral cortex.[8,9] Preclinical investigations have shown that the inhibition of cGMP-specific phosphodiesterases (PDEs) improves synaptic plasticity and enhances performance in spatial memory tasks.[4,10] On the other hand, pharmacological inhibition of neuronal nitric oxide synthase (nNOS), which lowers the production of NO and the signaling of cGMP downstream, has been shown to negatively impact memory in different cognitive models.[2,6,11]

PF-04447943 is a selective PDE9 inhibitor that increases brain cGMP levels by inhibiting PDE9A from functioning properly. Preclinical findings indicate cognitive-enhancing potential; however, clinical outcomes have been inconsistent.[8,9] The current study sought to examine whether the inhibition of PDE9 may alleviate spatial memory deficits caused by the nNOS inhibitor 7-nitroindazole (7-NI). The radial arm maze was used to test spatial learning and memory. This is a well-known way to test working and reference memory in mice that relies on the hippocampus.[5,12]

Therefore, the aim of the present study was to investigate the effects of the selective phosphodiesterase type 9 (PDE9) inhibitor PF-04447943 on spatial memory impairment induced by the neuronal nitric oxide synthase (nNOS) inhibitor 7-nitroindazole (7-NI) in mice. The study was conducted using the radial arm maze test to evaluate whether PDE9 inhibition could improve cognitive deficits associated with disruption of the nitric oxide/cyclic guanosine monophosphate (NO/cGMP) signaling pathway.

MATERIALS AND METHODS

Ethical Approval: The study was approved by the Kocaeli University Animal Experiments Local Ethics Committee (KOU-HADYEK) **(**Date: 31.01.2024, decision No: KOU-HADYEK 1/5-2024**).** All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and complied with national and international guidelines for the ethical use of laboratory animals.

Animals: A total of 50 male BALB/c mice (7–8 weeks old), weighing 20–30 g, were used in this study. The animals were obtained from the Istanbul Medipol University Medical Research and Experimental Animal Research Unit (MEDİTAM). Mice were housed in groups of five per cage at the X University Experimental Medicine and Research Unit (DETAB) animal laboratory under controlled environmental conditions (22 ± 2 °C; 55–75% humidity) with a 12-hour light/dark cycle (lights on at 08:00). Mice were food-deprived for 24 hours before testing, while water was provided ad libitum, as previously described in behavioral studies evaluating memory performance.[12] All experiments were conducted two weeks after arrival to allow acclimatization. Behavioral testing was performed between 08:00 and 16:00.

Drug Administration**:** Animals were randomly divided into five groups (n = 10 per group): Control, PF-04447943 (1 mg/kg), PF-04447943 (3 mg/kg), 7-nitroindazole (7-NI; 5 mg/kg), and 7-NI (5 mg/kg) + PF-04447943 (3 mg/kg). The dose of 7-NI (5 mg/kg) was selected based on previous studies demonstrating its impairing effects on memory performance.[2,6,11,12] Similarly, dosing regimens and intraperitoneal administration protocols were adapted from earlier behavioral studies investigating PDE inhibitors and NO pathway modulators.[12] PF-04447943 (1–3 mg/kg) was dissolved in sterile water and administered intraperitoneally (i.p.) 40 minutes before testing. 7-NI was dissolved in distilled water with dimethyl sulfoxide (DMSO) using a sonifier, as previously described,[6,11] and

administered intraperitoneally (i.p.) 40 minutes before behavioral testing. All drugs were freshly prepared on the day of the experiment and administered at a volume of 2 ml/kg.

Radial Arm Maze Test: The apparatus consisted of a central platform with eight arms extending radially outward. During habituation, mice were placed in the center and allowed to explore the maze for 15 minutes. The RAM test consisted of four trials, each lasting 15 minutes, with 30-minute inter-trial intervals, in accordance with previously reported protocols.[12] A small piece of wheat was placed at the end of each arm to motivate exploration. At the beginning of each trial, animals were positioned in the center of the maze. The first entry into an arm was not considered an error, whereas re-entry into a previously visited arm was defined as a working memory error. The number of errors and latency (time to complete the task, seconds) were recorded separately for each trial. The number of errors and latency (time to complete the task, seconds) were recorded separately for each trial. Behavioral scoring was performed by the investigator, who was aware of the treatment groups.

Statistical Analysis: Normality and homogeneity of variance were assessed to determine whether parametric or non-parametric statistical tests were appropriate. Since the data met parametric assumptions, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied. In the radial arm maze test, the number of incorrect arm entries and the time required to complete the task (latency, seconds) were expressed as mean ± standard error of the mean (SEM). Group comparisons were performed using one-way ANOVA and Tukey's post hoc test. In all statistical analyses, $p < 0.05$ was considered statistically significant.

RESULTS

When the number of errors made in the final trial of the radial arm maze test was evaluated, there was a significant difference among all groups [$F(4,45)=13.15$; $p<0.0001$]. 3 mg/kg PF-04447943 significantly reduced the number of errors (*$p<0.05$) compared to the control group, and 5 mg/kg 7-NI significantly increased the number of errors (**$p<0.001$) compared to the control group. Comparing the 5 mg/kg 7-NI + 3 mg/kg PF-04447943 (combination) group with the 5 mg/kg 7-NI group, it was found that the number of errors decreased significantly (***$p<0.0001$) (Figure 1).

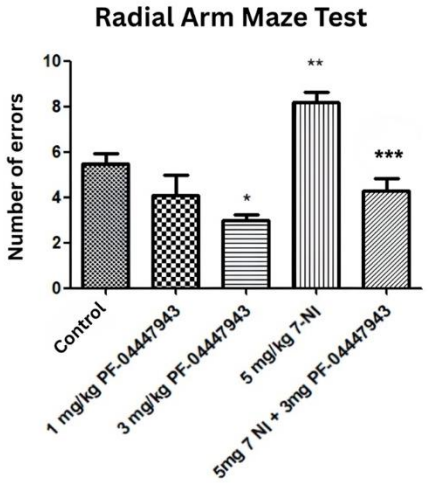


Figure 1. Number of working memory errors recorded during the fourth (final) trial of the radial arm maze test. Data are expressed as mean ± SD; *: $p < 0.05$ and **: $p < 0.001$ versus the control group; ***: $p < 0.0001$ versus the 7-NI: 7-Nitroindazole group.

When latency (time required to complete the task) in the 4th trial of the radial arm maze test was evaluated, a significant difference among groups was observed [$F(4,45) = 29.77$; $p < 0.0001$]. PF-04447943 (3 mg/kg) significantly reduced latency (*$p < 0.05$ vs. control), whereas 7-NI (5 mg/kg) significantly increased latency (***$p < 0.0001$ vs. control). Combined treatment (7-NI + PF-04447943) significantly reduced latency compared with the 7-NI group (**$p < 0.0001$) (Figure 2).

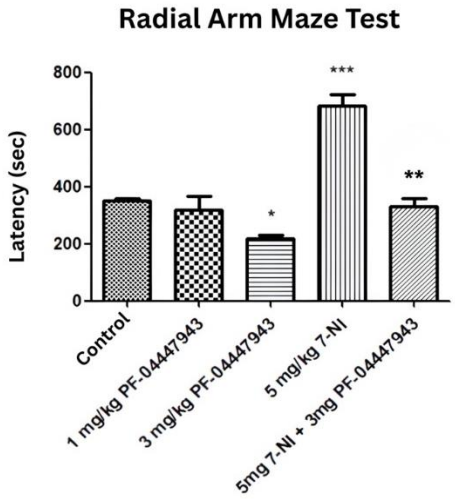


Figure 2. Latency (time required to complete the task) during the fourth (final) trial of the radial arm maze test. Data are expressed as mean ± SD; *: $p < 0.05$ and ***: $p < 0.0001$ versus the control group; **: $p < 0.0001$ versus the 7-NI: 7-Nitroindazole group.

The key data for the number of errors and latency in the fourth trial are summarized in Table 1 as mean ± SD, together with the corresponding statistical comparisons.

Table 1. Effects of PF-04447943 and 7-NI on working memory errors and latency during the fourth trial of the radial arm maze test (n = 10 per group).

Group	Number of errors (mean ± SD)	p value*	Latency (sec) (mean ± SD)	p value*
Control	5.5 ± 0.4	n/a	350 ± 20	n/a
PF-04447943 (1 mg/kg)	4.1 ± 0.9	n/a	320 ± 50	n/a
PF-04447943 (3 mg/kg)	3.0 ± 0.3	**<0.05**	220 ± 20	**<0.05**
7-NI (5 mg/kg)	8.2 ± 0.5	**<0.001**	680 ± 40	**<0.0001**
7-NI (5 mg/kg) + PF-04447943 (3 mg/kg)	4.3 ± 0.5	**<0.0001**	330 ± 30	**<0.0001**

7-NI: 7-nitroindazole; SD: standard deviation; n/a: not applicable; *: One-way ANOVA followed by Tukey's post hoc test.

The changes in working memory performance across the four consecutive trials of the radial arm maze test are shown in Figure 3. Figure 3A shows the changes in the number of working memory errors, which generally decreased over successive trials in all experimental groups. Figure 3B shows the changes in task completion time (latency), which also tended to decrease with successive trials in all experimental groups.

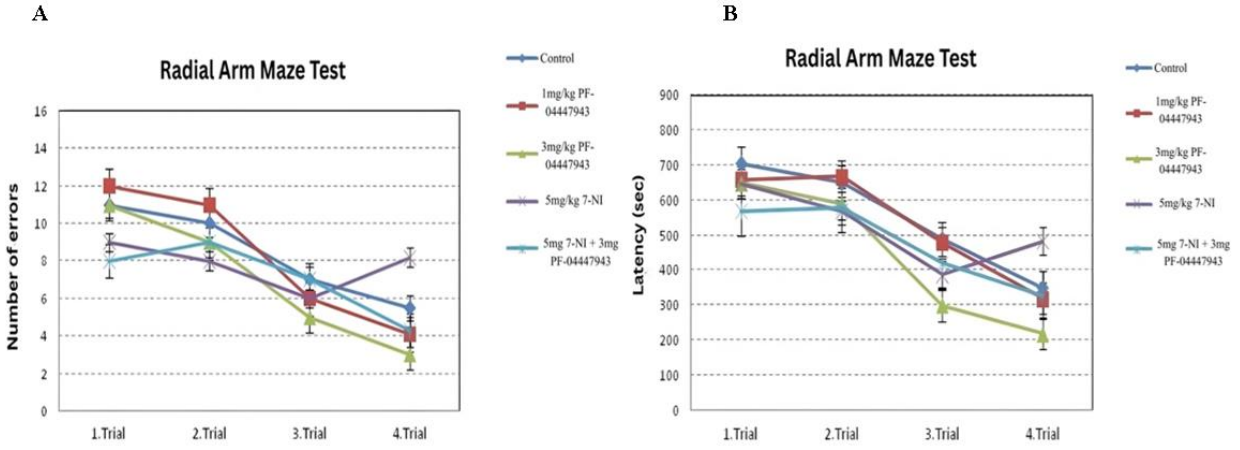


Figure 3. Changes in working memory performance during the four consecutive trials of the radial arm maze test. A: Changes in the number of working memory errors; B: Changes in task completion time (latency); Data are expressed as mean ± SD; 7-NI: 7-nitroindazole.

DISCUSSION AND CONCLUSION

The findings of the current study suggest that selective inhibition of phosphodiesterase type 9 (PDE9) improves spatial memory in mice and reduces cognitive problems linked to nitric oxide. These findings also support the important role of the NO/cGMP signaling pathway in hippocampal-dependent learning and memory. Recent studies have further strengthened the association between PDE9 activity, cGMP signaling, synaptic plasticity, and memory, supporting the biological relevance of this pathway to cognitive processes.[13–17]

PDE9 is a highly selective cGMP-hydrolyzing phosphodiesterase that has attracted increasing attention as a potential target for cognitive disorders because of its role in regulating intracellular cGMP signaling in the central nervous system.[13–15,18,19] Recent pharmacological studies have provided additional evidence supporting the cognitive relevance of PDE9 inhibition. In particular, a late study demonstrated that the selective PDE9 inhibitor irsenontrine (E2027) increased intracellular cGMP, enhanced GluA1 phosphorylation, increased hippocampal cGMP levels, and improved learning and memory in rodents.[16] The current study demonstrated that PF-04447943 significantly enhanced spatial memory performance at a dosage of 3 mg/kg, suggesting that selective inhibition of PDE9 may similarly enhance cognitive function through modulation of cGMP signaling.

Earlier studies have shown that activation of the cGMP/PKG pathway promotes LTP and memory consolidation through mechanisms including CREB phosphorylation, AMPA receptor trafficking, and synaptic remodeling. [14–16,18–21] More recent evidence has reinforced the role of the NO/cGMP pathway in synaptic plasticity and memory and has highlighted its interaction with CREB-dependent mechanisms.[16,17] Therefore, PDE9 inhibition may enhance these processes by reducing cGMP degradation and prolonging cGMP-dependent signaling. The recent finding that PDE9 inhibition increases cGMP and GluA1 phosphorylation further supports the possibility that modulation of cGMP may influence glutamatergic synaptic mechanisms relevant to learning and memory.[16]

Furthermore, PF-04447943 partially reversed the memory deficits induced by 7-NI, suggesting that PDE9 inhibition may compensate, at least partially, for reduced NO-dependent signaling by increasing cGMP availability. This is consistent with evidence implicating the NO/cGMP pathway in synaptic plasticity and memory.[15–17] However, the incomplete recovery suggests that PDE9 inhibition cannot fully replace intact NO signaling, which may influence cognition through mechanisms beyond cGMP metabolism. Thus, optimal memory formation likely depends on coordinated NO/cGMP/PKG, glutamatergic, and other intracellular pathways.

Alterations in cyclic nucleotide signaling have been implicated in cognitive decline and neurodegenerative diseases, highlighting PDEs as potential therapeutic targets.[14–17,19,20] However, the promising preclinical effects of PDE9 inhibition have not consistently translated into clinical efficacy, indicating a translational gap that may reflect differences in disease stage, treatment duration, pharmacokinetic properties, and patient characteristics.[16,19,20,23] Therefore, the present findings support PDE9/cGMP signaling as an experimental target rather than evidence of established therapeutic efficacy in Alzheimer's disease.

The present findings should also be considered in the context of different PDE9 inhibitors. PF-04447943 has been investigated in both experimental and clinical studies, while newer compounds such as BI 409306 and E2027 have provided further evidence on PDE9 modulation.[16,22–24] Although preclinical studies generally suggest cognitive and synaptic benefits, clinical outcomes have been less consistent.[19,23] Further studies are therefore needed to determine the mechanisms and conditions under which PDE9 inhibition may provide meaningful cognitive benefits.

A limitation of the present study is that molecular markers underlying the behavioral effects were not evaluated. In particular, hippocampal cGMP levels, PKG activity, CREB phosphorylation, and electrophysiological measures of synaptic plasticity were not assessed. In addition, behavioral scoring was not performed under blinded conditions, as the investigator recording the behavioral outcomes was aware of the treatment groups, which may have introduced observer bias. Future studies integrating behavioral, biochemical, and electrophysiological approaches are warranted to provide a more comprehensive evaluation of the therapeutic potential of PDE9 inhibition in cognitive impairments and Alzheimer's disease.[13–17,19,20]

In conclusion, selective PDE9 inhibition with PF-04447943 improved spatial memory performance in the radial arm maze test and partially reversed cognitive deficits induced by 7-NI-mediated nNOS inhibition in mice. These findings strengthen the experimental evidence linking PDE9-dependent cGMP regulation to cognitive function and support further investigation of PDE9 inhibition as a potential therapeutic strategy for cognitive impairment.

Ethics Committee Approval: The study was approved Animal Experiments Local Ethics Committee (KOU-HADYEK) (Date: 31 January 2024, Decision by the Kocaeli University No: 1/5-2024). All experimental

procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals and complied with national and international guidelines for the ethical use of laboratory animals.

Conflict of Interest: No conflict of interest was declared by the authors.

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