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Evaluation of The Efficacy of Pitavastatin on Pain Response in Rats with Thermal Plantar and Dynamic Plantar Tests

Pitavastatinin Ratlarda Termal Plantar ve Dinamik Plantar Testler ile Ağrı Yanıtına Etkinliğinin Değerlendirilmesi

Selma YAMAN[1]
0000-0002-9301-9119
Duygun ALTINTAŞ AYKAN[2]
0000-0001-8224-4006
Tuba ÖZCAN METİN[3]
0000-0003-0624-026X

[1]Department of Biophysics, Kahramanmaraş Sütçü İmam University Faculty of Medicine, Kahramanmaraş, Türkiye

[2]Department of Pharmacology, Kahramanmaraş Sütçü İmam University Faculty of Medicine, Kahramanmaraş, Türkiye

[3]Department of Histology and Embryology, Kahramanmaraş Sütçü İmam University Faculty of Medicine, Kahramanmaraş, Türkiye

Corresponding Author
Sorumlu Yazar
Selma YAMAN
yamanselma@yahoo.com

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ABSTRACT

Aim: Pitavastatin (Pita) is a member of the statin family, a well-known hypolipidemic agent, and some statin members are effective in treating pain. This study aimed to evaluate the antinociceptive effects of Pita by measuring nociception in rats with different doses and durations.

Material and Methods: This study consisted of six groups including saline oral as a non-drug control, 1 mg/kg Pita single dose, 3 mg/kg Pita single dose, 1 mg/kg Pita for 14 days, 3 mg/kg Pita for 14 days, and 20 mg/kg diclofenac for 14 days. Thermal plantar and mechanical plantar tests were used to observe pain threshold changes. Correlations between Pita doses, durations, and behavioral pain responses were evaluated. The sciatic nerves were evaluated histopathologically.

Results: Pita showed a significant antinociceptive effect in the dynamic plantar test at doses of 1 mg/kg for 14 days by increasing the mechanical threshold from 25.43±4.79 g to 32.28±2.27 g (p=0.041) and 3 mg/kg for 14 days by increasing the mechanical threshold from 27.41±2.36 g to 34.35±2.58 g (p=0.039). Also, Pita increased the thermal latency from 8.95±1.28 s to 11.71±1.49 s in the thermal plantar test at a single dose of 3 mg/kg (p=0.004). Although the antinociceptive effects of Pita were proven in dynamic plantar and thermal plantar tests, these findings did not reach a significant level at histopathological evaluation.

Conclusion: These results suggest that Pita has an antinociceptive effect and when used in conjunction with the right dosage and timing, might be favored for the treatment of pain.

Keywords: Pain; pitavastatin; antiallodynic effect; antihyperalgesic effect.

ÖZ

Amaç: Pitavastatin (Pita), iyi bilinen bir hipolipidemik ajan olan statin ailesinin bir üyesidir ve bazı statin üyelerinin ağrı tedavisinde etkili olduğu gösterilmiştir. Bu çalışmada sıçanlarda farklı doz ve sürelerde nosisepsiyon ölçülerek Pita'nın antinosiseptif etkilerinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışma ilaçsız kontrol olarak salin oral, tek doz 1 mg/kg Pita, tek doz 3 mg/kg Pita, 14 gün boyunca 1 mg/kg Pita, 14 gün boyunca 3 mg/kg Pita ve 14 gün boyunca 20 mg/kg diklofenak olmak üzere altı gruptan oluşmaktadır. Ağrı eşiği değişikliklerini gözlemlemek için termal plantar ve mekanik plantar testler kullanıldı. Pita dozları, süreleri ve davranışsal ağrı tepkileri arasındaki korelasyonlar değerlendirildi. Siyatik sinirler histopatolojik olarak değerlendirildi.

Bulgular: Pita, 14 gün boyunca uygulanan 1 mg/kg dozunda mekanik eşiği 25,43±4,79 g'dan 32,28±2,27 g'a çıkararak (p=0,041) ve 14 gün boyunca uygulanan 3 mg/kg dozunda ise mekanik eşiği 27,41±2,36 g'dan 34,35±2,58 g'a çıkararak (p=0,039) dinamik plantar testte anlamlı bir antinosiseptif etki gösterdi. Ayrıca Pita, termal plantar testte 3 mg/kg tek dozda termal latansı 8,95±1,28 sn'den 11,71±1,49 sn'ye çıkardı (p=0,004). Dinamik plantar ve termal plantar testlerde Pita'nın antinosiseptif etkileri kanıtlanmış olmakla birlikte, bu bulgular histopatolojik değerlendirmede anlamlı bir düzeye ulaşmadı.

Sonuç: Bu sonuçlar Pita'nın antinosiseptif etkiye sahip olduğunu ve uygun doz ve zamanlama ile kullanıldığında ağrı tedavisinde tercih edilebileceğini düşündürmektedir.

Anahtar kelimeler: Ağrı; pitavastatin; antiallodinik etki; antihiperaljezik etki.

INTRODUCTION

Pain serves as a protective mechanism for living organisms through the activation of nociceptors (1). Despite ongoing scientific progress in comprehending the genetics, pathology, molecular biology, and neurophysiology associated with pain, the preventive measures for adverse conditions—including the precise identification of its source, alleviation, and detrimental impacts on the life process—remain inadequately understood (2).

It may indicate the potential for innovative and more efficacious methods for pain management, considering various factors, including the limitations of existing treatment modalities that fail to eradicate the pain symptom, exhibit adverse side effects, and carry a risk of dependency (3,4).

Statins are involved in the regulation of cholesterol synthesis by enhancing the levels of apolipoprotein A1 (Apo-A1) and inhibiting the activity of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a mechanism that was initially identified in 1976 (5,6). The use of statins has been associated with a decrease in both mortality and morbidity in patients with various cardiovascular diseases (7,8).

Statins, beyond their established role in lowering lipid levels, have demonstrated various biological, anti-inflammatory properties, modulation of cell proliferation, enhancement of endothelial function, and influences on coagulation and platelet activity, as indicated by several studies (9-12). Among these agents, pitavastatin (Pita) is particularly notable for its strong ability to inhibit the enzyme HMG-CoA reductase (13,14).

Systemic administration of statins has demonstrated efficacy in preclinical investigations, indicating a potential to inhibit the onset of mechanical allodynia and thermal hyperalgesia in various experimental animal models (15-19).

Despite the encouraging results regarding the use of statins for alleviating nerve pain in animal subjects, the underlying mechanisms that contribute to these beneficial effects remain inadequately understood. Furthermore, the specific impact of statins on pain thresholds and their effectiveness in the context of chronic versus acute pain conditions has yet to be thoroughly elucidated.

Therefore, this investigation aimed to demonstrate the effectiveness of Pita, a member of the statin family, on pain threshold. It is hoped that the findings obtained from this study will contribute to the first-line literature in revealing the antiallodynic, antihyperalgesic activity of Pita in neurological animal models. After demonstrating its effectiveness on pain threshold in the initial stage, this study will help determine whether Pita will be effective in complex neuropathic pain conditions.

MATERIAL AND METHODS

Animals and Experimental Design

A total of thirty-six adult male Wistar rats (250-260 g) from the local medical sciences experimental research center were used in the experiments. All experiments were conducted strictly with the National Institute of Health Guidelines for the Care, and Use of Laboratory Animals. Experimental protocols were approved by Kahramanmaraş Sütçü İmam University Animal Experiments Ethics Committee (2020/02-04, approval date: 27.02.2020). Rats were kept in their cages until the day of the experiment in animal housing rooms where sound insulation was provided, and temperature (22-24 °C) was controlled under a 12:12-h light/dark cycle (06:00 am to 06:00 pm) with 40-60% relative humidity. The rats were housed in hygienic stainless steel cages where they could comfortably continue feeding and water consumption.

Drugs and Chemicals

Pita, supplied by Abdi Ibrahim Drug Company (Istanbul, Turkey), was homogenously dissolved in tap water, and delivered to rats per oral (p.o.) with orogastric gavage. In the study protocol, 1 mg/kg, and 3 mg/kg administration doses of Pita were determined based on the basic literature (20,21). Diclofenac Na was obtained from Deva Holding (Istanbul, Turkey), and anti-inflammatory doses of diclofenac 20 mg/kg were based on doses that we observed in our previous studies (22).

Experimental Design and Groups

The rats were brought to the laboratory 14 days before and on the day of the experiment and kept for 20 minutes to adapt to the environment. To reduce extra stress factors on the rats, the same researcher carried out the drug applications each time, and care was taken to guarantee that there was no excessive distance between the place where the experiments were carried out and the colony. Since the experiment involved researcher-animal contact, and some animal practices, before the experiments rats were acclimated to the person, and system at least three times for 20-30 s for 3 days. Animals were allocated to six groups designed as follows. Control: saline oral (non-drug control), Pita 1mg1d: 1 mg/kg Pita single dose (low dose and short-term administration), Pita 3mg1d: 3 mg/kg Pita single dose (high dose and short-term administration), Pita 1mg14d: 1 mg/kg Pita for 14 days, (low dose and long-term administration), Pita 3mg14d: 3 mg/kg Pita for 14 days (high dose and long-term administration), and Diclofenac 20mg14d: 20 mg/kg diclofenac administration for 14 days (positive control). Before drug administrations, thermal plantar, and dynamic plantar pain assessments were protocoled to standardize pain responses across all groups. The treatment regimen started on the first day. Thermal and dynamic plantar pain tests were repeated on the second day of the treatment regimen for those who took the drug as a single dose, and on the fifteenth day for those who took the drug for 14 days (Figure 1).

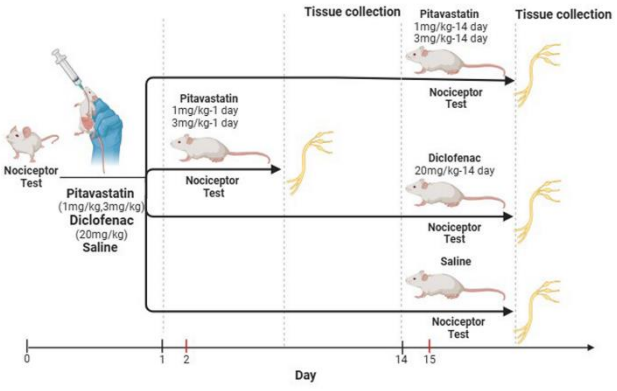

Figure 1. Experimental design of the groups

Nociception Test Procedures

Thermal Plantar Test

A thermal plantar test (Commat, Ankara, Turkey) was used to measure the latency of rats, which reflects the delay in paw withdrawal time in the presence of thermal hyperalgesia. Each animal was kept in plexiglass chambers for 10 minutes to adapt to the test environment in which they were previously placed. Under the glass plate on which the rats were placed, a manually moving radial heat source was appropriately held to give a thermal stimulus to the left/right posterior mid-plantar region. Thermal stimulus administration, 1% of the maximum intensity was used to focus the area of the hind paw. Stimulus intensity; the hind paw was adjusted to give a maximum of 25% infrared ray intensity, which would cause a total thermal latency of 8-10 s. The system is designed in such a way that when the rat pulls back its paw, which feels the pain that develops as a result of the stimulus, the infrared generator is automatically turned off via a photocell, and the timer displays the value that will determine the latency on the digital screen. The control mechanism of the system is designed to close in 25 seconds (cut-off latency) so that the animal cannot be exposed to too much stimulus when it does not retract its paw due to excessive desensitization (23).

Dynamic Plantar Test (Von Frey Filament Test)

Dynamic plantar aesthesiometer modified from a version of the von Frey filament test (Ugo Basile, Comerio, Italy), was used for the measurement of animal sensitivity to the non-noxious light mechanic touch of the paw. The mechanical allodynia measurement was determined by measuring the withdrawal threshold to a truly non-destructive mechanical stimulus applied to the rat's paw. Sudden withdrawals against mechanical stimuli were defined as mechanical allodynia (24).

Histopathological Evaluation

After being fixed in 10% buffered formalin, the sciatic nerve samples were prepared for paraffin block processing. Hematoxylin, and Eosin (H&E) staining was applied after the slices were cut at a thickness of 5 µm using a rotatory microtome. The images were examined under a Carl Zeiss Axio Imager A2 microscope at different magnifications. The sciatic nerve longitudinal sections were scored using the method described previously (25,26). In the histopathological evaluation, edema, vacuolization, mononuclear cellular infiltration, and axonal and myelin degenerations were considered and scored using a scale ranging from 0 to 3.

Statistical Analysis

For numerical data, Levene and Kolmogorov-Smirnov tests were employed to examine the variance homogeneity and normal distribution assumptions. The numerical data were defined with mean and standard deviation. Categorical variables were expressed as percentages and numbers. Repeated measures analysis of variance test was utilized to compare pre- and post-treatment values across multiple groups and post hoc Tukey and Bonferroni methods were used to examine in detail for the comparisons of pre- and post-treatment in each group. Fisher-Freeman-Halton test was used for categorical variables. The SPSS v.17.0 (SPSS Statistics for Windows, version 17.0. Chicago: SPSS Inc.) program was utilized for statistical analysis. The p-value of <0.05 accepted the criteria for statistical significance.

RESULTS

Nociception Test Result

The Effect of Diclofenac 20 mg/kg on Mechanical Threshold and Thermal Latency

Following the administration of diclofenac 20 mg/kg, the mechanical threshold after the mechanical stimulus, and thermal latency after the thermal stimulus were statistically significantly improved. Diclofenac 20 mg/kg significantly increased the mechanic threshold from 28.62±4.19 grams (g) to 37.62±4.31 g (p=0.009, Table 1). Diclofenac 20 mg/kg also markedly improved the thermal latency, which increased from 8.52±1.64 seconds (s) to 10.68±0.72 s (p=0.022, Table 2). Figures 2 and 3 also showed the alterations in thresholds and latencies caused by diclofenac 20 mg/kg.

The Effect of Pita 1 mg/kg on Mechanical Threshold and Thermal Latency

Pita 1 mg/kg for 14 days exerted a significant antinociceptive effect in the mechanical test. Pita 1 mg/kg for 14 days significantly increased the mechanical threshold from 25.43±4.79 g to 32.28±2.27 g (p=0.041). Mechanical and thermal plantar test results showed that a single dose of 1 mg/kg Pita did not reveal a significant improvement in pain responses (p=0.597, and p=0.287, respectively). The effects of Pita 1 mg/kg on paw withdrawal responses to mechanical stimuli were shown in Table 1 and Figure 2.

The Effect of Pita 3 mg/kg on Mechanical Threshold and Thermal Latency

Pita 3 mg/kg for 14 days exerted a significant antinociceptive effect in the mechanical test. Pita 3 mg/kg for 14 days increased the mechanical threshold from 27.41±2.36 g to 34.35±2.58 g (p=0.039). The effects of Pita 3 mg/kg on the paw withdrawal responses to mechanical

Table 1. Pre- and post-treatment dynamic plantar test responses, mechanic threshold (g) of groups

Groups	Pre-treatment	Post-treatment	p
Control	26.51±3.16	28.45±2.21	0.550
Pita 1mg1d	31.58±7.50	29.87±5.38	0.597
Pita 3mg1d	27.93±10.37	33.29±3.69	0.105
Pita 1mg14d	25.43±4.79	32.28±2.27	**0.041**
Pita 3mg14d	27.41±2.36	34.35±2.58	**0.039**
Diclofenac 20mg14d	28.62±4.19	37.62±4.31	**0.009**

Pita: pitavastatin, g: grams, data were reported as mean±standard deviation

Table 2. Pre- and post-treatment thermal plantar test responses, thermal latencies (s) of groups

Groups	Pre-treatment	Post-treatment	p
Control	9.22±0.41	9.14±0.63	0.931
Pita 1mg1d	9.31±2.24	10.28±1.82	0.287
Pita 3mg1d	8.95±1.28	11.71±1.49	**0.004**
Pita 1mg14d	8.33±0.53	8.48±1.44	0.868
Pita 3mg14d	9.69±1.39	9.42±1.80	0.766
Diclofenac 20mg14d	8.52±1.64	10.68±0.72	**0.022**

Pita: pitavastatin, s: seconds, data were reported as mean±standard deviation

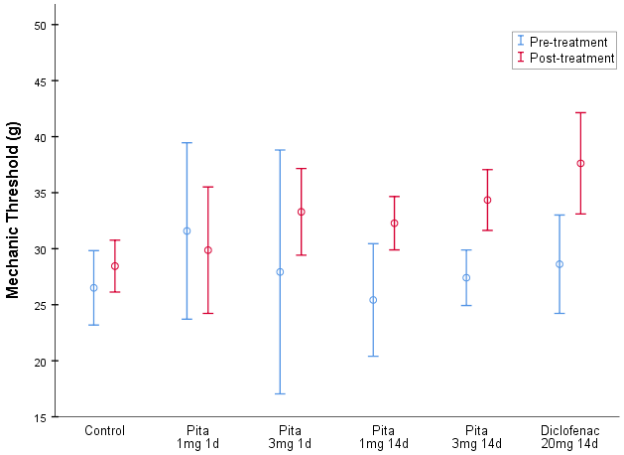

Figure 2. Pita at different doses and durations showed its effectiveness on mechanical allodynia with a significant increase in post-treatment mechanical threshold (g) in groups of Pita 1mg14d, Pita 3mg14d, and diclofenac 20mg14d compared to pretreatment values

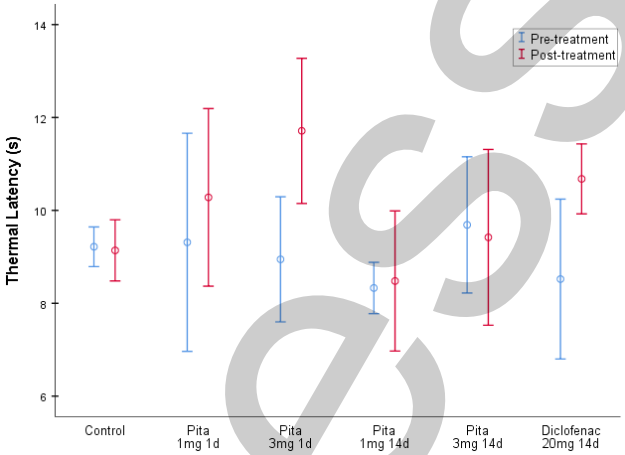

Figure 3. Pita at different doses and durations showed its effectiveness on thermal hyperalgesia with a significant increase in post-treatment thermal latencies (s) in groups of Pita 3mg1d and diclofenac 20mg14d compared to pretreatment values

stimuli were shown in Table 1, and Figure 2. In addition, thermal plantar test results showed that a single dose of Pita 3 mg/kg revealed a significant improvement in pain responses. Pita 3 mg/kg single dose enhanced the thermal latency from 8.95±1.28 s to 11.71±1.49 s (p=0.004). The effects of Pita 3 mg/kg on the paw withdrawal responses to the thermal stimuli were shown in Table 2 and Figure 3.

Histopathological Results

Histopathological examination showed that the sciatic nerves of the control group had a normal histoarchitecture with well-organized myelin sheath, and axons (Figure 4a). Conversely, Pita 1mg1d, Pita 3mg1d, Pita 1mg14d, Pita 3mg14d, and diclofenac 20mg14d groups exhibited degenerated myelinated axons, with separated distributed nerve fibers, mononuclear cellular infiltration, vacuolization as well as edema (Figure 4b, 4c, 4d, 4e, 4f). Diclofenac 20mg14d group sections showed the worst histological picture (Figure 4f). However, when the score results of these parameters were evaluated, no significant difference was seen between the experimental study, and control groups (Table 3).

DISCUSSION

Statins are classified as inhibitors of HMG-CoA reductase and are employed in the management of conditions characterized by elevated cholesterol levels (27). In addition to their ability to reduce cholesterol levels, statins are recognized for a variety of other beneficial properties, including anti-inflammatory effects and pain-relieving capabilities (28,29). The results of this investigation indicate that Pita may exhibit an antinociceptive effect, as evidenced by its impact on both thermal and mechanical thresholds, which were assessed through thermal and mechanical plantar test responses administered at the appropriate dosage and timing.

The selection of diclofenac as the positive control group was based on its recognized analgesic effectiveness and the alignment of test outcomes with findings from prior studies (22). Allodynia and hyperalgesia are recognized as manifestations of sensitization occurring in both peripheral and central processes (30,31). Peripheral and central sensitization processes may potentially be connected to neurochemical alterations and neuroinflammatory consequences in the pain perception process. Studies suggest that statins may possess anti-neuroinflammatory properties (32). Neuroinflammation is an immune system response with complex subcomponents that neural tissues

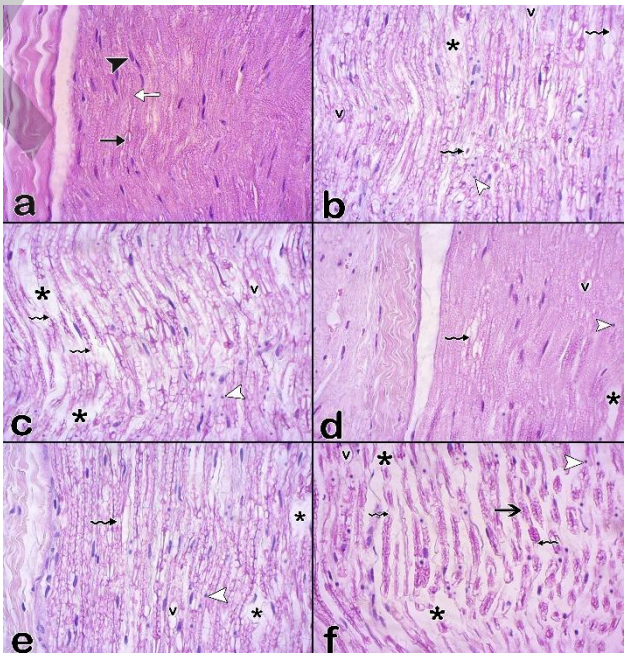

Figure 4. Effect of Pita on sciatic nerve histopathological changes, **a)** the control group showed normal axons (black arrow) with intact myelin sheaths (white arrow) and Schwann cell (arrowhead), **b)** Pita 1mg1d, **c)** Pita 3mg1d, **d)** Pita 1mg14d, **e)** Pita 3mg14d, **f)** Diclofenac 20mg14d groups showed axonal degeneration (wavy arrows), vacuolization (v), edema (asterisks) and mononuclear cell infiltration (white arrowheads), the diclofenac 20mg14d group also showed the fragmentation (black arrow) of myelin and axons (Hematoxylin, and Eosin (H&E), 400x)

Table 3. Comparison of histopathological evaluation results between groups

	Control	Pita 1mg1d	Pita 3mg1d	Pita 1mg14d	Pita 3mg14d	Diclofenac 20mg14d	p
Axonal Degeneration, n (%)							
Absent	3 (50.0%)	1 (16.7%)	2 (33.3%)	1 (16.7%)	1 (16.7%)	0 (0.0%)	0.437
Mild	3 (50.0%)	3 (50.0%)	1 (16.7%)	5 (83.3%)	4 (66.7%)	4 (66.7%)	
Moderate	0 (0.0%)	2 (33.3%)	2 (33.3%)	0 (0.0%)	1 (16.7 %)	1 (16.7%)	
Severe	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	
Infiltration, n (%)							
Absent	4 (66.7%)	2 (33.3%)	2 (33.3%)	3 (50.0%)	2 (33.3%)	1 (16.7%)	0.877
Mild	2 (33.3%)	4 (66.7%)	3 (50.0%)	3 (50.0%)	3 (50.0%)	4 (66.7%)	
Moderate	0 (0.0%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	1 (16.7%)	1 (16.7%)	
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Vacuolization, n (%)							
Absent	3 (50.0%)	2 (33.3%)	1 (16.7%)	2 (33.3%)	2 (33.3%)	0 (0.0%)	0.478
Mild	3 (50.0%)	2 (33.3%)	3 (50.0%)	4 (66.7%)	3 (50.0%)	5 (83.3%)	
Moderate	0 (0.0%)	2 (33.3%)	2 (33.3%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	
Edema, n (%)							
Absent	4 (66.7%)	1 (16.7%)	1 (16.7%)	3 (50.0%)	3 (50.0%)	2 (33.3%)	0.540
Mild	2 (33.3%)	4 (66.7%)	3 (50.0%)	2 (33.3%)	1 (16.7%)	1 (16.75)	
Moderate	0 (0.0%)	1 (16.7%)	2 (33.3%)	1 (16.7%)	2 (33.3%)	3 (50.0%)	
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Myelin Degeneration, n (%)							
Absent	4 (66.7%)	2 (33.3%)	2 (33.3%)	3 (50.0%)	2 (33.3%)	2 (33.3%)	0.984
Mild	2 (33.3%)	3 (50.0%)	2 (33.3%)	2 (33.3%)	3 (50.0%)	2 (33.3%)	
Moderate	0 (0.0%)	1 (16.7%)	2 (33.3%)	1 (16.7%)	1 (16.7%)	1 (16.7%)	
Severe	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	

can exhibit during various processes (33). In previous studies, simvastatin and atorvastatin have been reported to exhibit antihyperalgesic, and antiallodynic effects, as well as antinociceptive consequences in animal models of neuropathic pain (18,34).

In this research, the antiallodynic effect demonstrated statistical significance in the groups receiving Pita at dosages of 1 mg/kg for 14 days and 3 mg/kg for 14 days, particularly in relation to mechanical allodynia, according to data from a modernized von Frey application employed in plantar mechanical responses in the assessment of nociception. However, Pita in short-term dosages (1 mg/kg single dose and 3 mg/kg single dose) is not as effective as long-term administration.

The C fiber group, comprising polymodal nociceptors, is responsible for responding to temperature changes in nociception. The C fiber group, which includes polymodal nociceptors, plays a crucial role in detecting variations in temperature as part of the nociceptive process. The current study's thermal plantar test responses with C fiber supplementation revealed that Pita significantly prolonged thermal latency in the 3 mg/kg single dose treatment group compared to the pretreatment condition.

While there was a relative improvement in thermal delay reductions in the other dose-time application groups, this did not reach statistical significance. This finding is consistent with the effect of Pita on thermal hyperalgesia in the partial sciatic nerve-induced neuropathy model (35). Previous studies have mentioned that the antinociceptive effect may occur through several regulatory effects such as inflammatory pathways, and oxidative stress (28,36).

Atorvastatin has been shown to have neuroinflammatory activity in the ischemic stroke model (1 or 10 mg/kg) and in the chronic constriction-induced nerve injury model (3, 10, and 30 mg/kg by oral gavages for 14 days) (28,37).

If neuropathic pain is to be evaluated from a different perspective, another important relationship between neuropathic pain and RhoA/Rho kinase stands out. Both RhoA and Rho-related kinase (Rho kinase) play important roles in the maintenance of chronic pain states (38). In another study, simvastatin reduced nociceptive behaviors by blocking p38 mitogen-activated protein kinase, and microglial RhoA (39). Statins have been also shown to have an inhibitory effect on the Rho/Rho kinase signaling pathway (40). This phenomenon can also be considered as an example of a pleiotropic effect, which refers to the lipid-independent actions of statins, including their capacity to inhibit Rho-Rho kinase activity. In this context, it seems plausible that the antinociceptive properties of Pita may affect pain thresholds even in the absence of a neuropathic disorder.

In the study investigating the potential effects of a group of analgesic drugs on the sciatic nerve, more severe effects were observed with diclofenac compared to the other drugs such as lornoxicam, morphine, and pethidine groups (25). Similarly, in the diclofenac group in the present study, separated nerve fibers, mononuclear cellular infiltration, vacuolization, edema, and degenerated myelinated axons were reported based on the histopathological evaluation of their effects on the sciatic nerve. Additionally, reflex behaviors including withdrawal thresholds against heat, and pressure noxious stimuli for pain sensitivity measurement, were examined in this study, and histopathological evaluation consistency with the diclofenac compared to the control group.

This research has some limitations. Firstly, the effectiveness of Pita on pain has not been discussed with a neuropathy model. Secondly, the study methodology did not include molecular techniques such as assessing inflammatory markers. It is necessary to add a detail here

that, under initial conditions, the study was planned in two stages; the initial instance is to reveal the effectiveness of Pita on pain threshold within the framework of dose-time data, and in the second stage; it is planned to support this effect with experimental neuropathy models.
In subsequent research, it would be beneficial to incorporate and analyze Pita treatment protocols across various animal models, alongside the molecular findings observed. The findings from this investigation demonstrate the antiallodynic and antihyperalgesic properties of Pita, as evidenced by thermal and dynamic plantar tests conducted at varying dosages and time intervals, complemented by histological analysis. Additional research is required to clarify the mechanisms through which Pita exerts its antinociceptive effects, particularly in relation to different molecular markers within neuropathy models.

CONCLUSION

In this study, Pita was shown to have antiallodynic and antihyperalgesic effects at different doses, and times with thermal, and dynamic plantar tests, and histological data. These results suggest that Pita has an antinociceptive effect and may be preferred for pain management. Further studies are needed to clarify how Pita's antinociceptive activity mediates its role in pain pathology through various molecular markers on neuropathy models.

Ethics Committee Approval: The study was approved by the local ethics committee on animal experiments at Kahramanmaraş Sütçü İmam University (27.02.2020, 04).

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REFERENCES

1. Muley MM, Krustev E, McDougall JJ. Preclinical assessment of inflammatory pain. CNS Neurosci Ther. 2016;22(2):88-101.
2. Max MB, Stewart WF. The molecular epidemiology of pain: a new discipline for drug discovery. Nat Rev Drug Discov. 2008;7(8):647-58.
3. Jain KK. Gene therapy for pain. Expert Opin Biol Ther. 2008;8(12):1855-66.
4. Meert TF, Vermeirsch HA. A preclinical comparison between different opioids: antinociceptive versus adverse effects. Pharmacol Biochem Behav. 2005;80(2):309-26.
5. Lennernäs H, Fager G. Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors. Similarities and differences. Clin Pharmacokinet. 1997;32(5):403-25.
6. Wasim R, Ansari TM, Ahsan F, Siddiqui MH, Singh A, Shariq M, et al. Pleiotropic benefits of statins in cardiovascular diseases. Drug Res (Stuttg). 2022;72(9):477-86.
7. Sirtori CR. The pharmacology of statins. Pharmacol Res. 2014;88:3-11.
8. Mantell G. Lipid lowering drugs in atherosclerosis--the HMG-CoA reductase inhibitors. Clin Exp Hypertens A. 1989;11(5-6):927-41.
9. Chen XY, Li K, Light AR, Fu KY. Simvastatin attenuates formalin-induced nociceptive behaviors by inhibiting microglial RhoA and p38 MAPK activation. J Pain. 2013;14(11):1310-9.
10. Grundy SM. Statin trials and goals of cholesterol-lowering therapy. Circulation. 1998;21;97(15):1436-9.
11. Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme reductase inhibitors. Arterioscler Thromb Vasc Biol. 2001;21(11):1712-9.
12. Bhalla S, Singh N, Jaggi AS. Statins: do they aggravate or ameliorate neuropathic pain? J Pain. 2014;15(11):1069-80.
13. Hoy SM. Pitavastatin: A review in hypercholesterolemia. Am J Cardiovasc Drugs. 2017;17(2):157-68.
14. Duggan ST. Pitavastatin: a review of its use in the management of hypercholesterolaemia or mixed dyslipidaemia. Drugs. 2012;72(4):565-84.
15. Shi XQ, Lim TKY, Lee S, Zhao YQ, Zhang J. Statins alleviate experimental nerve injury-induced neuropathic pain. Pain. 2011;152(5):1033-43.
16. Bhalla S, Singh N, Jaggi AS. Dose-related neuropathic and anti-neuropathic effects of simvastatin in vincristine-induced neuropathic pain in rats. Food Chem Toxicol. 2015;80:32-40.
17. Cameron N, Cotter M, Inkster M, Nangle M. Looking to the future: diabetic neuropathy and effects of rosuvastatin on neurovascular function in diabetes models. Diabetes Res Clin Pract. 2003;61(Suppl 1):S35-9.
18. Pathak NN, Balaganur V, Lingaraju MC, More AS, Kant V, Kumar D, et al. Antihyperalgesic and anti-inflammatory effects of atorvastatin in chronic constriction injury-induced neuropathic pain in rats. Inflammation. 2013;36(6):1468-78.
19. Pan HC, Yang DY, Ou YC, Ho SP, Cheng FC, Chen CJ. Neuroprotective effect of atorvastatin in an experimental model of nerve crush injury. Neurosurgery. 2010;67(2):376-89.
20. Iqbal R, Akhtar MS, Hassan MQ, Jairajpuri Z, Akhtar M, Najmi AK. Pitavastatin ameliorates myocardial damage by preventing inflammation and collagen deposition via reduced free radical generation in isoproterenol-induced cardiomyopathy. Clin Exp Hypertens. 2019;41(5):434-43.
21. Hu G, Ito O, Rong R, Sakuyama A, Miura T, Ito D, et al. Pitavastatin upregulates nitric oxide synthases in the kidney of spontaneously hypertensive rats and Wistar-Kyoto rats. Am J Hypertens. 2018;31(10):1139-46.

22. Altintas Aykan D, Yaman S. Evaluation of the effects of tadalafil on pain response in thermal plantar and dynamic plantar tests in rats. Ann Med Res. 2020;27(3):749-54.
23. Mert T, Metin TO, Sahin E, Yaman S, Sahin M. Neuroprotective and anti-neuropathic actions of pulsed magnetic fields with low frequencies in rats with chronic peripheral neuropathic pain. Brain Res Bull. 2021;177:273-81.
24. Mert T, Metin TO, Sahin M, Yaman S. Antiinflammatory properties of antiLy6G antibody disappear during magnetic field exposure in rats with carrageenan induced acute paw inflammation. Naunyn Schmiedebergs Arch Pharmacol. 2020;393(11):2107-15.
25. Balaha M, Kandeel S, Kabel A. Phloretin either alone or in combination with duloxetine alleviates the STZ-induced diabetic neuropathy in rats. Biomed Pharmacother. 2018;101:821-32.
26. Bostan H, Cabalar M, Altinay S, Kalkan Y, Tumkaya L, Kanat A, et al. Sciatic nerve injury following analgesic drug injection in rats: A histopathological examination. North Clin Istanb. 2018;5(3):176-85.
27. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: an update. Fundam Clin Pharmacol. 2005;19(1):117-25.
28. Jung KH, Chu K, Jeong SW, Han SY, Lee ST, Kim JY, et al. HMG-CoA reductase inhibitor, atorvastatin, promotes sensorimotor recovery, suppressing acute inflammatory reaction after experimental intracerebral hemorrhage. Stroke. 2004;35(7):1744-9.
29. Chu LW, Chen JY, Yu KL, Cheng KI, Wu PC, Wu BN. Neuroprotective and anti-inflammatory activities of atorvastatin in a rat chronic constriction injury model. Int J Immunopathol Pharmacol. 2012;25(1):219-30.
30. Viana F. Nociceptors: thermal allodynia and thermal pain. Handb Clin Neurol. 2018;156:103-19.
31. Weber MS, Prod'homme T, Steinman L, Zamvil SS. Drug insight: using statins to treat neuroinflammatory disease. Nat Clin Pract Neurol. 2005;1(2):106-12.
32. Skaper SD, Facci L, Zusso M, Giusti P. An inflammation-centric view of neurological disease: beyond the neuron. Front Cell Neurosci 2018;12:72.
33. Corso CR, Martins DF, Borges SC, Beltrame OC, Telles JEQ, Buttow NC, et al. Effect of simvastatin on sensorial, motor, and morphological parameters in sciatic nerve crush induced-neuropathic pain in rats. Inflammopharmacology. 2018;26(3):793-804.
34. Goel R, Kumar N, Kumar Saxena P, Pratap Singh A, Bana S. Pitavastatin attenuates neuropathic pain induced by partial sciatic nerve in Wistar rats. J Pharm Pharmacol. 2023;75(1):66-75.
35. Pathak NN, Balaganur V, Lingaraju MC, Kant V, Latief N, More AS, et al. Atorvastatin attenuates neuropathic pain in rat neuropathy model by down-regulating oxidative damage at peripheral, spinal and supraspinal levels. Neurochem Int. 2014;68:1-9.
36. Jung KH, Chu K, Jeong SW, Han SY, Lee ST, Kim JY, et al. HMG-CoA reductase inhibitor, atorvastatin, promotes sensorimotor recovery, suppressing acute inflammatory reaction after experimental intracerebral hemorrhage. Stroke. 2004;35(7):1744-9.
37. Chen XY, Li K, Light AR, Fu KY. Simvastatin attenuates formalin-induced nociceptive behaviors by inhibiting microglial RhoA and p38 MAPK activation. J Pain. 2013;14(11):1310-9.
38. Tatsumi S, Mabuchi T, Katano T, Matsumura S, Abe T, Hidaka H, et al. Involvement of Rho-kinase in inflammatory and neuropathic pain through phosphorylation of myristoylated alanine-rich C-kinase substrate (MARCKS). Neuroscience. 2005;131(2):491-8.
39. Gillon JT, Smith SE, Lowden MR. Atorvastatin as novel treatment for neuropathic pain: a case report. Clin J Pain. 2013;29(12):e46-8.
40. Nohria A, Prsic A, Liu PY, Okamoto R, Creager MA, Selwyn A, et al. Statins inhibit Rho kinase activity in patients with atherosclerosis. Atherosclerosis. 2009;205(2):517-21.