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Original Article

Evaluation of the effects of tadalafil on pain response in thermal plantar and dynamic plantar tests in rats

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Abstract

Aim: Nitric oxide and its promoters, phosphodiesterase-related agents, have been demonstrated to have pivotal roles in pain modulation. Phosphodiesterase-5 (PDE5) inhibitors are important to enhance the effect of endogenously released nitric oxid. In this study, we aimed to evaluate the tadalafil efficacy, a PDE5 inhibitor, in central nociception models in rats.

Material and Methods: Thirty-six rats were divided into six treatment groups. Mechanic plantar aesthesiometer and thermal plantar tests were employed to measure the pain thresholds to the mechanical and thermal stimulations. Correlations between the tadalafil doses, durations and behavioral pain responses were recorded, and compared with that of diclofenac, a nonsteroidal anti-inflammatory drug.

Results: Tadalafil 1 and 10 mg/kg single doses; and tadalafil 1 and 10 mg/kg for 7 days exerted significant antinociceptive effects on the mechanic plantar aesthesiometer. However, tadalafildid not reveal significant amelioration in pain responses on the thermal plantar test.Tadalafil 1 mg/kg caused an insignificant amelioration in thermal latencies and withdrawal thresholds in comparison to 10 mg/kg doses.

Conclusion: Our findings indicated that nociceptive effect of tadalafil due to thermal stimulation involves cyclic guanosine monophosphate (cGMP), while in mechanic hyperalgesiac GMP may not have a basic role in the primary sensory neurons sensitization. The increase in latencies and withdrawal thresholds with low dose tadalafil was remarkabl.

Keywords: Tadalafil; phosphodiesterase 5 inhibitors; nociception; pain; pain measurement

INTRODUCTION

Pharmacological management of pain includes inhibition of cyclooxygenases, suppression of pain signals and inhibition of nociceptor sensitivity. For this purpose, non-steroidal anti-inflammatory drugs (NSAIDs) exert their mechanisms of action by the inhibition of the synthesis of cyclooxygenases. Accordingly,opioids and nitric oxide (NO) donors suppress pain signals and inhibit nociceptive sensitivity (1). NO is produced by the NO synthase enzyme isoform and is associated with acute and chronic pain response in central and peripheral nervous system. The intracellular targets, which NO is associated,have been well-documented as the guanylatecyclase enzyme activation, an increase in the level of cyclic guanosine monophosphate (cGMP), and the modulation of cGMP-dependent protein kinase and ATP-sensitive potassium channels (2).According to these data, agents that promote NO production or inhibit its degradation may cause an analgesic effect.

Tadalafil, a novel therapeutic agent in the management of erectile dysfunction, is a potent, reversible and competitive inhibitor of phosphodiesterase 5 (PDE5). It inhibits the degradation of cGMP which acts mainly through activated protein kinase G (PKG). PDE5 inhibitors are effective through the NO-cGMP pathway, and are important to enhance the effect of endogenously released NO (3).

Spesific PDE inhibitors have been studied for analgesic or hyperalgesic effects in various pain experiments to promote NO levels. Sildenafil, cGMP specific PDE5 inhibitor, has been shown to exhibit a protective role for neuronal cells via the mitochondrial K+ATP channels-dependent mechanisms (4), while another PDE5 inhibitor, vardenafil, activates the NO-cGMP / calcium channels (5). In fact, tadalafilis more selectivefor PDE5 than other

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inhibitors, has faster onset within 1 hour, and has longer action duration up to 36 hours (6). Tadalafil was reported to have anti-inflammatory effects by the amelioration of cytokines and chemokines in the circulation (7). Another study demonstrated the local effects of tadalafil in the reduction of pelvic pain and inflammation (8). Analgesic effect of tadalafil was provided via guanylyl cyclase pathway and was associated with a reduction in neutrophils and tumor necrosis factor-α level (9).

The current study was undertaken to investigate the effect of tadalafil on the nociceptive pain responses,with different pain measurement tests, different durations and doses, and to compare these effects with a NSAID,diclofenac.

MATERIAL and METHODS

Experimental Animals and Housing Conditions

A total number of thirty-six Wistar albino rats, weight 200-300 g, were utilized in this study. Housing was achieved in hygienic stainless steel cages with free access to food and tap water. Rats were kept at an ambient temperature of 22°C and 60±5% humidity with a 12-hr light/dark cycle throughout the experiment, and access to water and food ad libitum. Acclimatization for the study conditions was allowed for one week prior to the start of the study. All experiments were conducted in strict accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. Experimental protocols were approved by the Local Animal Experimentation Ethics Committee (File No: 2019/03/05, Approval date: 08.05.2019).

Drugs and Chemicals

Tadalafil, supplied from Abdi Ibrahim Drug Company (Istanbul, Turkey), was homogenously dissolved in tap water and delivered to rats per oral (p.o.) with orogastric gavage. In humans, tadalafil treatment was recommended as 5-10 mg daily up to 20 mg. In animal studies, the doses were administered as 1.6 mg/kg (equals to 20 mg in adult human),and 0.45 mg/kg in rats (equals to 5 mg in adult human). Hence, in our protocols, we analyzed the effects of tadalafil on nociceptive pain response by administering 1 mg/kg and 10 mg/kg,based on the fundamental literature (10,11).

Diclofenac was purchased from Deva Holding A.Ş. (Istanbul, Turkey), was prepared at the concentration of 20 mg/kg, which has shown analgesic effects according to our previous assays and based on the literature (12,13), and administered p.o. by means of an orogastric gavage once a day for 7 days.

Experimental Design

Thirty-six Wistar albino rats were subjected to mechanical stimulus (mechanical plantar test) and thermal stimulus (thermal plantar test) prior to the drug administrations to analyze the basal pain responses. The rats were then divided randomly into six groups (n = 6). The groups were carried out as follows:

Group I received saline for 7 days;

Group II received tadalafil 1 mg/kg as single dose;

Group III received tadalafil 10 mg/kg as single dose;

Group IV received tadalafil 1 mg/kg for 7 days;

Group V received tadalafil 10 mg/kg for 7 days;

Group VI received diclofenac 20 mg/kg for 7 days.

Pain measurements were protocoled after 1 day post-drug treatment in order to standardize pain responses in all groups. Hence, in group II and group III, nociception responses were evaluated on the 2nd day. Group I, group IV, group V and group VI received the tadalafil treatment for 7 days, hence, pain measurements were analyzed on the 8th day.

Assessment of Tadalafil Efficacy in Central Nociception Models

Mechanical Plantar Test (Von Frey Filament Test)

Mechanical paw withdrawal threshold was performed on hind paws using automated version of von Frey filament test, electronic mechanic plantar aesthesiometer (UgoBasile, Comerio, Italy). Mechanical allodynia is the brisk paw withdrawal in response to mechanical stimulus due to significant decrease in threshold, as described in our previous study (12). Rats were placed in individual plexiglass boxes on a stainless steel mesh floor. A force of 2.5 g/s was applied via a 0.5 mm diameter straight metal filament to the plantar surface of hindpaw until animal lifts its foot. The mechanical withdrawal threshold was the pressure exerted in grams that triggered the paw withdrawal. Mechanical stimulus was automatically turned off to avoid tissue damage when 50 g cut-off force was reached. Each stimulus was applied 3 times, and results from each hind paw were averaged. Baseline withdrawal thresholds were determined in all animals before any experimental procedure, and 2 and 8 days after the tadalafil administrations.

Thermal Plantar Test

Thermal plantar test was used to quantify heat thresholds in the hind paws of rats upon application of an infrared heat stimulus, as described in our previous study(12). To acclimatize to the testing environment, animals were allowed at least 10 min in plexiglass chambers (10 cm x 20 cm x 24 cm) on a clear glass platform. A radiant heat source was placed underneath the rat, aiming at the plantar surface of the hind paw. Thermal stimulus was delivered to the mid plantar region of right or left hind paws through a radiant heat source mounted on a movable holder below a glass pane. When the rat feels pain and withdraws its paw, infrared generator is turned off automatically, and timer stops, determining the withdrawal latency. The time taken to withdraw from the heat stimulus was recorded as the withdrawal latency automatically by the apparatus (Commat, Ankara, Turkey). The paw withdrawal latencies were measured by a digital watch electrically connected with the heat source. The

thermal plantar test permits measurement of ipsilateral and contralateral heat thresholds. A 25 s cut-off time was imposed on the stimulus duration to avoid any tissue injury. The paw withdrawal latencies were tested 3 times for each hindpaw, and results from each hind paw were averaged. Baseline withdrawal latencies were determined in all animals before any experimental procedure, and 2 and 8 days after the tadalafil administrations.

Statistical Analysis

Data was defined as arithmetic mean and standard deviation. Before applying parametric tests, Kolmogorov Smirnov test was used to determine the suitability of the data for normal distribution and homogeneity of the variances. Repeated sample test and Wilcoxon signed-rank test were used for the analysis of dependent data. For the evaluation of multiple groups, variance test analysis with post-hoc Tukey's test was used in normally-distributed data. For the non-normally distributed data, Kruskal Wallis test with Mann Whitney U test under Bonferroni correction was used. The $p<0.05$ were considered significant. Data was evaluated at 95% confidence interval.

RESULTS

The Effect of Diclofenac on Mechanic and Heat Hypersensitivity

The thermal latency after thermal stimulus and the mechanical threshold after mechanical stimulus were significantly improved following administration of diclofenac 20 mg/kg. Diclofenac significantly enhanced the thermal latency from 9.43±0.98 seconds (s)to 11.78±1.37 s (p=0.008) as shown in Table 1. In addition, diclofenac caused a significant increase in mechanic threshold from 25.40±4.45 grams (g) to 31.38±3.76 g (p=0.032) asdemonstrated in Table 2. The alterations in latencies and thresholds produced by diclofenac were also demonstrated in Figures 1 and 2.

Table 1. Effects of tadalafil and diclofenac treatments on the thermal latencies at thermal plantar test

Variable	Pretreatment	Posttreatment	p
Saline	9.82±1.00	10.30±0.43	0.270
TAD 1 mg/kg_1day	10.87±1.09	11.95±2.20	0.329
TAD 10 mg/kg _1day	10.02±0.93	11.10±1.27	0.169
TAD 1 mg/kg _7days	9.83±1.03	10.88±0.80	0.172
TAD 10 mg/kg _7days	10.42±1.22	10.41±0.88	0.980
DIC	9.43±0.98	11.78±1.37[a]	0.008

[a], denotes significant increase in thermal latency in DIC group compared to pretreatment values."p<0.05" and the "±" is standard deviation. TAD: Tadalafil, DIC: Diclofenac

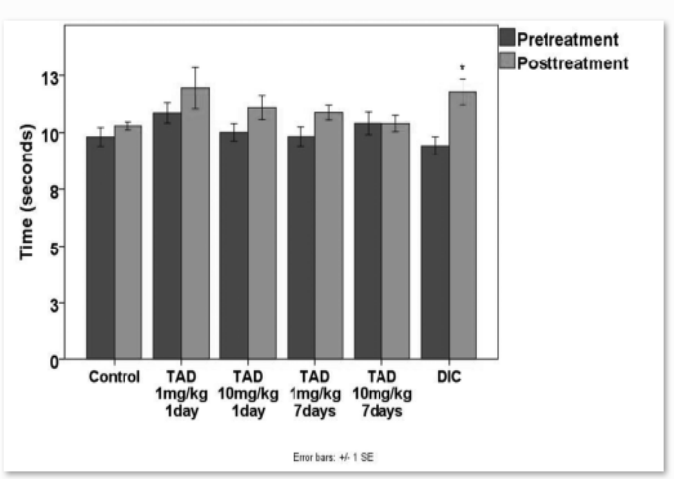

Figure 1: *denotes significant increase in thermal latency in DIC group compared to pretreatment values (p=0.008). TAD: Tadalafil, DIC: Diclofenac, Control: Saline

The Effect of Treatment with Tadalafil 1 mg/kg on Mechanic and Heat Hypersensitivity

Tadalafil 1 mg/kgas single dose and tadalafil 1 mg/kg for 7 days exerteda significant antinociceptive effect in mechanical test. Our results showed that tadalafil 1 mg/kgas a single dosesignificantly increased the mechanical threshold from 28.06±5.65 g to 38.37±3.78 g (p=0.01), and tadalafil 1 mg/kg for 7 days increased the mechanical threshold from 22.13±5.30 g to 30.80±5.01 g (p=0.007). However, tadalafil 1 mg/kg for any period did not reveal a significant amelioration in pain responses in the thermal plantar test. In addition, tadalafil 1 mg/kg caused an insignificant amelioration in thermal latencies and withdrawal thresholds in comparison to 10 mg/kg doses. Table 1 and Figure 1 showed the influence of tadalafil 1 mg/kg on the paw withdrawal responses to the thermal stimuli. Mechanical stimuli produced by tadalafil 1 mg/kg were demonstrated in Table 2 and Figure 2.

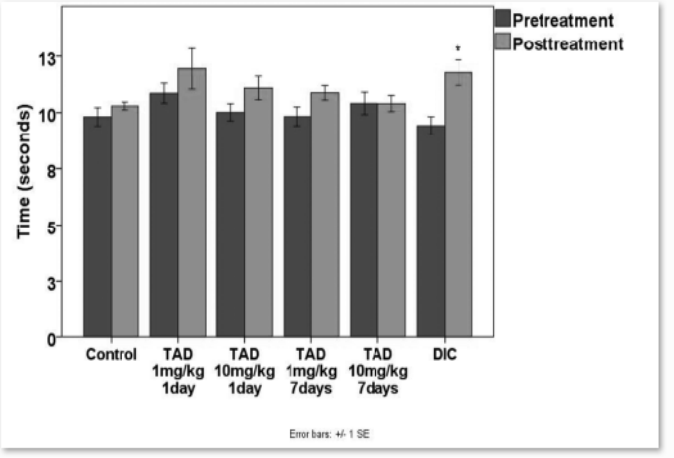

Figure 2. *denotes significant increase in mechanical threshold in groups of TAD 1mg/kg_1day, TAD 10mg/kg _1day, TAD 1mg/kg _7days, TAD 10mg/kg _7days, and DIC; compared to pretreatment values (p= 0.010, <0.001, 0.007, 0.038, 0.032 respectively). TAD: Tadalafil, DIC: Diclofenac, Control: Saline

The Effect of Treatment with Tadalafil 10 mg/kg on Mechanic and Heat Hypersensitivity

Administration of tadalafil 10 mg/kg as a single dose and tadalafil 10 mg/kg for 7 days produced a significant antinociceptive effect in mechanical test. Tadalafil 10 mg/kg as a single dose significantly enhanced threshold from 24.53+3.86 g to 36.33+4.82 g($p<0.01$), and tadalafil 10 mg/kg for 7 days significantly enhanced threshold from 23.93+4.14 g to 29.48+3.70 g ($p=0.038$). However, tadalafil 10 mg/kg for any period did not reveal an enhancement in the thermal plantar test results.Table 1 and Figure 1 showed the influence of tadalafil 10 mg/kg on the paw withdrawal responses to the thermal stimuli. Mechanical stimuli produced by tadalafil 10 mg/kg were demonstrated in Table 2 and Figure 2.

Table 2. Effects of tadalafil and diclofenac treatments on the mechanic threshold at dynamic plantar test

Variable	Pretreatment	Posttreatment	p
Saline	23.99±3.87	24.93±2.24	0.530
TAD 1 mg/kg_1day	28.06±5.65	38.37±3.78[a]	0.010
TAD 10 mg/kg _1day	24.53±3.86	36.33±4.82[b]	<0.001
TAD 1 mg/kg _7days	22.13±5.30	30.80±5.01[c]	0.007
TAD 10 mg/kg _7days	23.93±4.14	29.48±3.70[d]	0.038
DIC	25.40±4.45	31.38±3.76[e]	0.032

[a,b,c,d,e], denote significant increase in thermal latency in groups of TAD 1 mg/kg_1day, TAD 10 mg/kg _1day, TAD 1 mg/kg _7days, TAD 10 mg/kg _7days, and DIC; compared to pretreatment values. "p<0.05" and the "±" is standard deviation.
TAD: Tadalafil, DIC: Diclofenac

DISCUSSION

We investigated the antinociceptive effect of tadalafil treatment with dose dependent manner (1 mg/kg and 10 mg/kg, for 1 day and 7 days) in rats, and compared these effects with diclofenac,a NSAID analgesic. Rats were subjected to the two different measurements of central nociception involving thermal and mechanical stimuli. We found that tadalafil 1 and 10 mg/kg single doses; and tadalafil 1 and 10 mg/kg for 7 days exerted significant antinociceptive effects on the mechanical test. However, tadalafil 1 mg/kg or 10 mg/kg for any period did not reveal a significant amelioration in pain responses on the thermal plantar test.

In our study, reflex behaviors involving withdrawal thresholds against noxious stimuli such as heat and pressure were evaluated to examine pain mechanisms. These measurements have been proven useful in advancing physiological basis of nociception, pharmacological and non-pharmacological pain treatments, intracellular pathways of neurotransmission, receptor identification, and genes involved in pain behaviors (14). Further, the pharmacological action of analgesics in animal studies should be consistent with human analgesia (15). As already known, traditional NSAIDs and opioids are commonly used in the management of pain-related symptoms. Hence, in the present study, we used diclofenac, a well-known NSAID, as a positive control in comparison of pain responses. As expected, the administration of diclofenac 20 mg/kg caused a significant increase both in the thermal latency and the mechanical threshold.

Reflective pain tests are known to evaluate the evoked behavior responses subsequent noxious stimuli such as heat, cold, mechanic, and electric, which are raised from the activation of nociceptors and result in reflexive motor responses (16). In the current study, in thermal and mechanical plantar tests, the paw withdrawal latencies and the mechanical withdrawal thresholds were tested three times on the bilateral hindpaws. Results from the bilateral hind paws were recorded and averaged. Many of these responses are modified by supraspinal sites. Changes in thresholds or latencies after noxious stimuli at the site of injury are defined as primary hyperalgesia and are responsible for sensitization of nociceptive primary afferents. Secondary hyperalgesia is involved in sensitization of neurons in the spinal cord or higher in the central nervous system (15). The method of von Frey filaments detects cutaneous hyperalgesia or allodynia by measuring mechanical withdrawal thresholds, and imitating neuropathic pain, postoperative pain, inflammation or osteoarthritis,which are reported as clinical conditions with cutaneous sensitivity (17,18).

Tadalafil blocks the degradation of cGMP which acts mainly through activated PKG. PDE5 inhibitors are effective through the NO-cGMP pathway, and are important to increase the NO effect (3). The role of NO in the inflammation has been well-documented at peripheral and central nervous system; however, the findings are still controversial. In fact, although there are different experimental models that explained the nociceptive effect of NO, many studies have documented the antinociceptive effects of this molecule (19).

Neuronal balance of adenosine monophosphate (cAMP) and cGMP is important in the modulation of primary sensory neuron sensitivity in mechanical stimulation. Our results revealed that tadalafil treatment at the doses of 1 mg/kg and 10 mg/kg caused significant antinociceptive effects with longer withdrawal duration against the noxious mechanical stimuli on the mechanical plantar test when compared to the pretreatment values. However, tadalafil treatment with doses of 1 mg/kg and 10 mg/kg did not cause significant ameliorations in the thermal latencies in comparison to the pretreatment values in thermal plantar test. The possible explanation may be that tadalafil may increase the nociceptive response to

the thermal stimulation because it causes a clear increase in cGMP at the spinal level (6). On the other hand, cGMP may not be pivotal in the mechanical test. In accordance, protein kinase A (PKA) was documented toinduce increased response level in the spinothalamic tract cells to the mechanical noxious stimulus, thus exerted an effect on acute pain (20). The results are in accordance to the earlier report which suggested that nociception in response to the thermal stimulation involves cGMP at this site. Supportingly, in mechanical hyperalgesia, cAMP was reported to play a primary role by the activation of adenylyl cyclase in the spinal cord and sensitization of primary sensory neurons (4). Peripheral activation of cAMP/PKA pathway has been demonstrated to utilize pain-like responses, and secondary hyperalgesia induced by capsaicin and cAMP analogs were decreased by the PKA inhibition (21).

In the current study we used low (1 mg/kg) and high doses (10 mg/kg) of tadalafil to inhibit PDE. We found an insignificant improvement in thermal latencies and mechanic withdrawal thresholds with 1 mg/kg doses. Similarly, in a previous study, bucladesin (Db-cAMP), a cyclic nucleotide derivative and phosphodiesterase inhibitor which mimics the effect of endogenous cAMP, was employed. Brito et al reported that low doses of Db-cAMP lowered the sensation of pain, and higher doses triggered nociception due to direct sensitization of nociceptors. They also reported that highlevels of intracellular cAMP in resident cells reduced inflammatory cytokine release and thus resulted in antinociception, while alike high levels of cAMP at nociceptor terminals triggered pain (22). Salehi et al suggested that Db-cAMP at low doses, similarly, showed antinociceptive effect by inhibiting inflammatory cytokine release; however, at higher doses, direct sensitization of nociceptors or activation of PKA have been proven more efficient (20).

CONCLUSION

In conclusion, we showed that tadalafil caused significant antinociceptive effect on mechanical test, but not on thermal plantar test. In addition, the insignificant increase in thermal latencies and withdrawal thresholds with low dose of tadalafil was remarkable.Our findings indicated that nociceptive effect of tadalafil due to thermal stimulation involves cGMP, while in mechanical hyperalgesia cGMP may not play a pivotal role in sensitization of the primary sensory neurons.

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