Tadalafil Preserves Penile Nitric Oxide Synthase from Detrimental Effect of Paroxetine in Rats

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ABSTRACT

Objective: Paroxetine is a commonly prescribed SSRI that can impair erectile function in animal models via inhibition of nitric oxide synthase (NOS). Tadalafil potentiates nitric oxide (NO)-mediated responses in isolated trabecular smooth muscle and penile erection. The purpose of this study was to evaluate the impact of co-administering tadalafil with paroxetine on penile NOS levels in rats.

Materials and Methods: A total of 30 male Sprague-Dawley rats were divided into 3 groups as control (Group-C), paroxetine (Group-P) and paroxetine plus tadalafil (Group-P+T). After 28 days of treatment, rats were sacrificed and their penile tissues were harvested for analysis. NOS isoform protein levels and immunoreactivity scores of NOS were assessed. Statistical significance level was set at $p<0.05$.

Results: Neuronal NOS (nNOS) levels were significantly decreased in group-P, compared with group-C ($p<0.001$). In comparison, rats in group-P+T had significantly higher nNOS levels compared to group-P ($p<0.001$). Endothelial NOS (eNOS) and inducible NOS (iNOS) levels were significantly higher in group-P compared with group-C ($p<0.01$). The levels of eNOS and iNOS in group-P+T were similar to group-C.

Conclusion: Daily treatment with tadalafil prevented chronic paroxetine-induced changes in all three NOS isoform levels. Tadalafil treatment may therefore be a useful therapy in men with paroxetine-associated erectile dysfunction.

Keywords: Erectile dysfunction, tadalafil, paroxetine, nitric oxide, rat

Introduction

Antidepressants drugs are frequently prescribed. While major depressive disorder is the original intended target of antidepressant medications, they have been increasingly used off label to manage obsessive-compulsive disorder, generalized anxiety disorder, other psychiatric conditions, and premature ejaculation [1]. Selective serotonin reuptake inhibitors (SSRIs) result in increased serotonin levels by preventing reuptake of this neurotransmitter. However, SSRIs are associated with side effects including erectile dysfunction (ED), which affects up to 50% of men using SSRIs [2]. Not all SSRIs result in ED at comparable frequencies. Namely, paroxetine is an SSRI that is associated with a high frequency of ED [3].

Previous studies have linked paroxetine's inhibition of nitric oxide synthase (NOS) with its propensity to cause ED [4, 5]. There are three types of NOS: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). NOS catalyzes the synthesis of nitric oxide (NO) from L-arginine, and NO activates guanylate cyclase enzyme, resulting in increased cyclic guanosine monophosphate (cGMP) levels inside the endothelial smooth muscle cells. These events facilitate penile corporal smooth muscle relaxation, increase blood flow into the corpora cavernosa, and consequently result in a firm erection [6].

Phosphodiesterase type 5 (PDE5) inhibitors are commonly used to treat ED. Like other phosphodiesterase inhibitors, the PDE5 inhibitors prevent cGMP hydrolysis, leading to higher cGMP levels. Uniquely, PDE5 is localized to the corpus cavernosum, and thus PDE5 inhibitors facilitate erection without significant systemic vasodilation [7]. Tadalafil is a highly effective PDE5 inhibitor, with even low daily doses improving erectile function in up to 86% of patients [8].

Paroxetine can decrease the NOS levels, and tadalafil works in the NO cascade to increase levels of downstream products. Therefore, concomitant administration of the two drugs might mitigate

the paroxetine-dependent changes in the NOS levels. Here, we present data examining eNOS, iNOS, and nNOS levels in penile tissue as a function of paroxetine and tadalafil treatment.

Materials and Methods

Animals

After obtaining Institutional Animal Care and Use Committee (IACUC approval no. 2014/12), 30 seven-week-old male Sprague-Dawley rats weighing 250-300 g were randomly allocated into three groups of 10 each. Rats were allowed unlimited water and fed standard chow, and they were kept at 22°C with 12 hours of light and 12 hours of dark per day. Daily for four weeks, the rats were orally administered medications between 1200 and 1400 hours. In the control group (Group C), rats were administered a placebo of 5ml/kg tap water. Group P was given 20 mg/kg paroxetine dissolved in 5ml/kg tap water. The paroxetine+tadalafil group (Group P+T) was given the paroxetine solution plus 5 mg/kg tadalafil (5 mg tadalafil dissolved in 1 mL tap water).

On the 29th day of the protocol, all rats were sacrificed. First, the rats were anesthetized using a combination of 5mg/kg xylazine, followed by 65 mg/kg ketamine administered intramuscularly 15 min later. Each rat was shaved, and it was placed in the supine position. A suprapubic vertical incision was used to access and excise the penis down to the level of the pubic symphysis. Subsequently, pentobarbital dosed at 150 mg/kg was administered. The distal one-third of the resected penile tissue was used for immunohistochemical analysis, whereas the proximal two-thirds were sectioned in phosphate buffered saline (PBS) in a petri dish placed on ice (NaCl-phosphate; 0.2M, pH 7.29) for Enzyme-Linked ImmunoSorbent Assay (ELISA) analysis.

Histopathological Analysis

The distal portions of the rat penis were fixed in 10% buffered formaldehyde solution for one day. Tissues were embedded in paraffin blocks, and 4 mm thick sections were made. Lysine-coated slides were prepared, and immunohistochemical analyses with nNOS (ab3511, polyclonal, IgG isotype, UK), iNOS (ab204017, polyclonal, IgG isotype, UK), and eNOS (ab50010, polyclonal, IgG isotype, UK) antibodies were performed. All antibodies were diluted with a 1:100 ratio. Sections were examined for staining intensity by a pathologist (SA) who was blinded to the rats' group. Semi-quantitative scoring was used where 0=none, 1=mild, 2=moderate, and 3=intense staining.

NOS Enzyme Levels

Cavernosal tissues were excised, and the urethral tissues were excluded. The cavernosal tissues were diced into small pieces in cold PBS and homogenized using a homogenizer (Ultra Turrax Type T25-B, IKE Labortechnic, Germany) at 16,000 rpm for 3 min on ice in buffer. Afterwards, they were rehomogenized with a glass homogenizer on ice, and then they were subjected to two freeze thaw cycles followed by ultrasonication. The resulting homogenates were centrifuged at 5000 rpm for 30 min at -20°C. Protein levels of NOS isoforms (nNOS, eNOS, and iNOS) were evaluated using commercial ELISA kits (Cloud-Clone Corp., Houston, TX) at 450 nm using a spectrophotometer (BioTek ELISA reader model, Winooski, VT) by using 100 µL homogenate. Results were calculated in ng/ml.

Statistical Analysis

Immunohistochemical data and differences in the NOS levels were examined using one-way analysis of variance and post-hoc Tukey test. All statistical analyses were performed using GraphPad Prizm 5 (GraphPad Software, Inc. La Jolla, CA). Results were expressed as mean±SD, with $p<0.05$ considered significant.

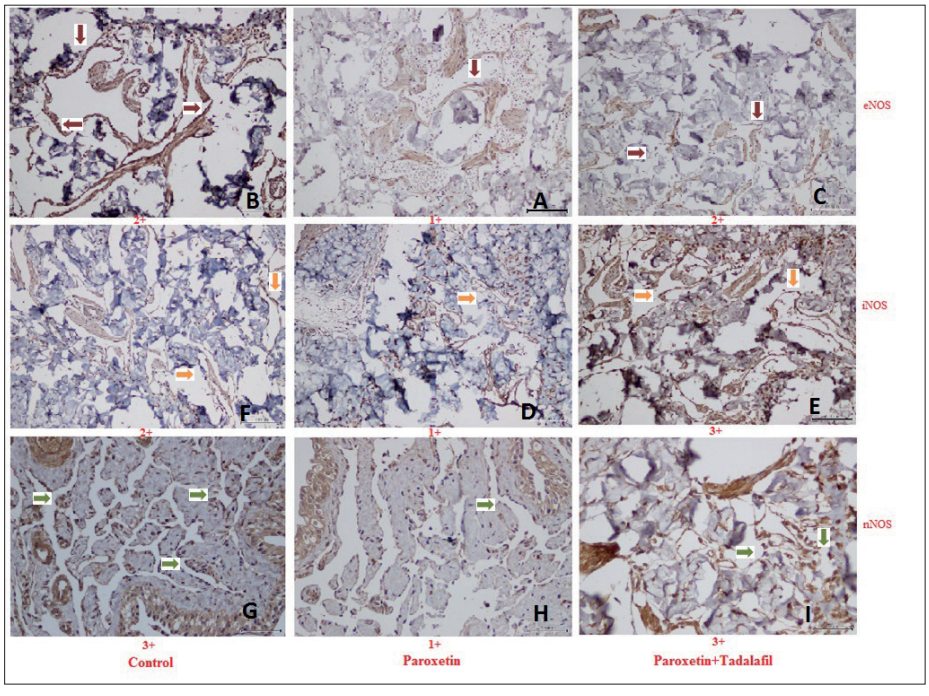

Figure 1. Immunohistochemical studies in the cavernosal tissue. Enhanced eNOS staining (red arrow) in cavernous sinus endothelial cells can be seen in Group P (B, x200). Moderate eNOS immunoreactivity is observed in the endothelial cells in Group P+T (C, x200). Similarly, strong iNOS staining (yellow arrows) is observed in Group P (E, x200) whereas less immunoreactivity was observed in Group P+T (Fx200). Finally, nNOS immunoreactivity was the greatest in the endothelial cells of the Group C (G, x400) (green arrows). Decrease in nNOS staining in the endothelial cells of Group P (H, x400) and enhanced nNOS staining in Group P+T can be observed (Ix400)

Results

Histopathological Analyses of NOS Levels in Corpus Cavernosum

Immunohistochemical analyses of corporal cavernosal tissues are shown in Figure 1. Semi-quantitative staining intensity scoring of cavernosal tissues revealed that the nNOS levels were significantly lower in Group P when compared to those in Group C (0.6±0.69 vs. 2.2±0.42, $p<0.001$), whereas co-administering tadalafil with paroxetine was associated with preserved nNOS levels in Group P+T (1.7±0.48) that was similar to those in Group C ($p=0.122$). Corporal eNOS levels were significantly higher for Group P compared to those in Group C (2.8±0.42 vs. 2.1±0.31, $p=0.003$), but there was no significant difference in eNOS levels between Group P+T (2.4±0.51) and each of Group C ($p=0.274$) and Group P ($p=0.109$). Moreover, the iNOS levels in Group P were significantly higher compared to those in Group C (2.6±0.51 vs. 1.9±0.56, $p=0.012$), whereas they were similar in Group C vs. Group P+T (1.9±0.56 vs. 2.2±0.42, $p=0.393$) and Group P vs. Group P+T (2.6±0.51 vs. 2.2±0.42, $p=0.199$).

ELISA Analyses NOS Levels in Penile Tissues

The NOS isoform protein levels in penile tissues were determined using ELISA (Table 1, Figure 2). Group P had significantly lower nNOS than Group C had ($p<0.001$). Simultaneously administering paroxetine with tadalafil was able to preserve the nNOS levels in Group P+T, which were significantly higher than those in Group P ($p<0.001$). Group P also had significantly higher eNOS ($p<0.01$) and iNOS ($p<0.01$) levels than

Table 1. The nNOS, eNOS, and iNOS levels of groups in ELISA

	nNOS			eNOS			iNOS		
	Group C	Group P	Group P+T	Group C	Group P	Group P+T	Group C	Group P	Group P+T
Mean	3.578	1.963	3.508	3.154	3.760	3.410	2.894	3.448	3.190
±SD	0.276	0.187	0.186	0.220	0.230	0.423	0.286	0.263	0.321

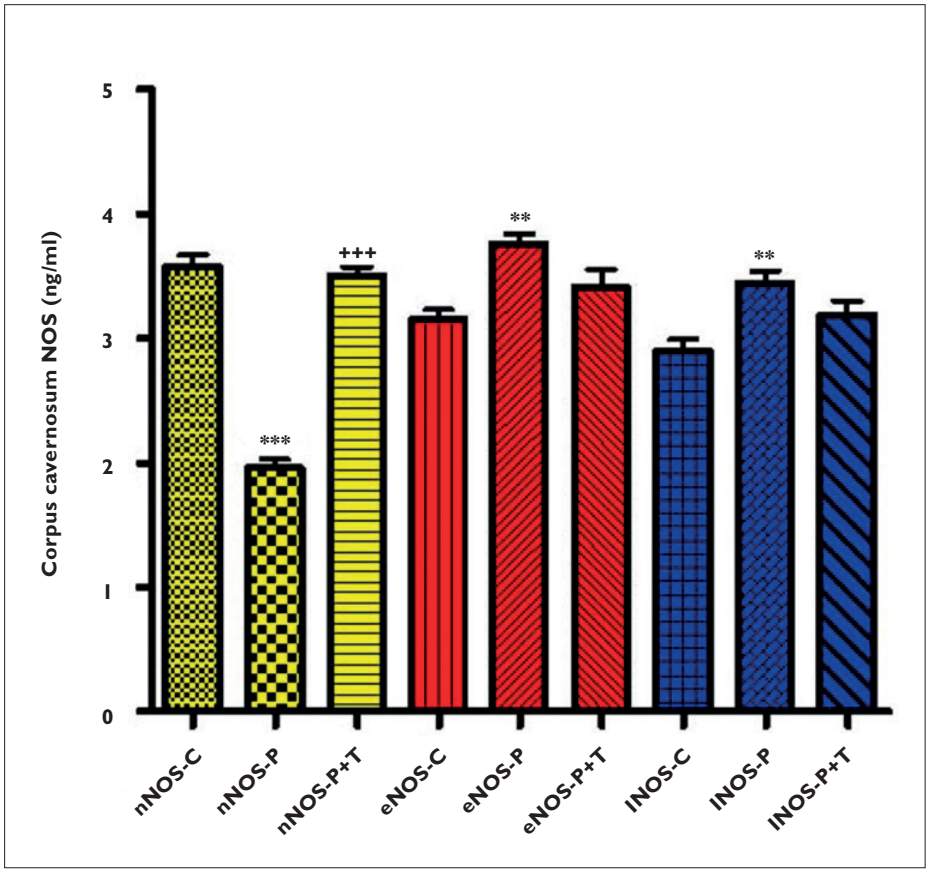

Figure 2. The NOS isoform levels measured by ELISA

Group C had. No difference in eNOS (p=0.122) and iNOS (p=0.226) levels was observed in Group P+T compared to the those in Group C.

Discussion

Each year in the United States, a growing population is treated for mental illnesses [9]. Therefore, there is a concomitant rise in the prescription and use of psychotropic medications, in particular antidepressants [10-12]. In 2010, there were 253.6 million antidepressant prescriptions totaling approximately $11 billion dollars in medication costs [13, 14]. However, the use of antidepressants involves side effects. In men, antidepressants can impair fertility and semen quality [15-18]. Moreover, numerous antidepressants (particularly SSRIs) can also dramatically impact libido and sexual function. Several animal studies have shown their negative effects on erectile function [5, 19-21].

The mechanism by which these drugs affect sexual function is incompletely understood. Both dopamine and serotonin are believed to be involved in sexual function, with dopamine having a protective effect and serotonin having an inhibitory effect [22]. However as all SSRIs increase serotonin levels similarly, serotonin levels alone cannot account for the sexual side effects of SSRIs, but some—such as paroxetine—have a much higher prevalence of sexual side effects. Moreover, increases in NAPDH oxidase or reactive oxygen species resulting in a decrease in NO bioavailability may also be responsible for SSRI-induced ED [23]. However, paroxetine seems to have a unique ability to inhibit NOS [5].

NOS, specifically nNOS, is a recent area of interest in erectile function studies [4, 24]. Contrary to predictions, nNOS-deficient rats had normal sexual and erectile function, which may be due to compensatory, protective overexpression of eNOS [4]. Angulo et al. [5] used cavernosal nerve electrical stimulation to create frequency-dependent intracavernosal pressure (ICP) increases. They found that the NOS inhibitors and acute and chronic paroxetine treatment all caused decreased ICP responses. In contrast, citalopram-treated rats responded similarly to how the rats in the control group did [5]. This is believed to be because of the structural similarity of paroxetine, but not citalopram, to the cytochrome P450 isoenzymes, which allows paroxetine to inhibit NOS [25, 26]. Similarly, chronic treatment with paroxetine, but not citalopram, caused a significant decrease in erectile response. Additionally, in the paroxetine-treated rats, plasma nitrite and nitrate levels were decreased by 61% ($p<0.05$), while the citalopram-treated rats had normal plasma nitrite and nitrate levels when compared to those in the controls. When examining protein levels in penile tissues, the nNOS levels were decreased by 31% in the paroxetine treatment group. The nNOS levels were not affected by citalopram treatment, and the eNOS levels did not significantly chang in either group. Thus, both acute and chronic paroxetine treatment may lead to an inhibition of nNOS, and therefore NO production causing a reduction in plasma NO derivatives and a decreased ICP response to cavernous nerve stimulation [5]. It is important to note that Angulo et al. [5] did not find a compensatory eNOS increase associated with decreased nNOS levels, as observed in other studies [4]. A possible explanation for this difference is that the rats lacked nNOS only after they were developmentally mature, and therefore they could not upregulate eNOS expression like nNOS knock-out rats did, which lacked the enzyme from conception [5]. On the other hand, the compensatory rise in eNOS could suggest an inhibitory function.

In our study, the nNOS levels decreased in the corpus cavernosum with paroxetine treatment, but combined treatment with tadalafil preserved the nNOS levels in both ELISA homogenates and immunohistochemical staining. Moreover, our analyses demonstrated an increase in eNOS and iNOS levels with paroxetine treatment, which is prevented by co-treatment with tadalafil. In a similar study, Kadioglu et al. [20] studied electrical field stimulation (EFS) induced relaxation of the penile tissues, which is mediated by NO. They found that both paroxetine and the NOS inhibitor L-NAME inhibited relaxation of penile tissue. The group hypothesized that paroxetine decreases NO production by acting as an inhibitor

of either eNOS or nNOS. On the other hand, sertraline and fluoxetine were found to increase EFS-induced relaxation of penile tissue via a relaxing factor—possibly NO [20]. The detrimental effect of paroxetine on erectile function has also been demonstrated in another study of Angulo et al. [27] who found that acute treatment with paroxetine significantly reduced ICP increase in response to cavernous nerve stimulation when compared with that in the control group. Conversely, vardenafil significantly potentiated the stimulation-dependent ICP increase compared to the control group. The authors recorded that administering paroxetine and a PDE5 inhibitor (vardenafil) together led to a similar ICP response as that seen in the control group [27].

This study has limitations. Comparing the ICP measurement outcomes among the three groups could support the therapeutic effect of daily tadalafil treatment in paroxetine-induced penile changes. Similarly, analyses of the phosphorylation status of NOS isoforms might reveal other mechanism associated with the impact of paroxetine and tadalafil on cavernosal tissues. Moreover, assessment of NOS isoforms in penile endothelial cell, smooth muscle cells, and neuronal cells separately by immunohistochemistry methods would provide a better insight about the extent of paroxetine-induced NOS alterations. Therefore, future molecular and further studies must be carried out to elucidate the mechanisms by which paroxetine and tadalafil affect NOS expression levels.

In conclusion, the increase in general psychological disorders and common use of SSRIs, namely paroxetine, is associated with sexual side effects, which include ED. Our data suggest that daily tadalafil may be a useful adjunct therapy, in concert with paroxetine, to prophylactically prevent unwanted sexual side effects and increase patient satisfaction with their treatment regimen in men with depression and premature ejaculation or other illnesses such as paraphilias and chronic pain syndromes.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Bagcilar Training and Research Hospital (IACUC approval no. 2014/12).

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Conflict of Interest: Authors have no conflicts of interest to declare.

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