Bedtime doses of prazosin do not affect daytime salivary amylase markers in PTSD

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ABSTRACT

Overactivity of the noradrenergic (NE) system within the central nervous system (CNS) has been postulated as a key pathophysiology of posttraumatic stress disorder (PTSD). The activity of the enzyme salivary α-amylase (sAA) has been proposed as an indirect measure of CNS NE activity, and sAA is elevated in PTSD. As an antagonist of the α-1 NE receptor, prazosin would be expected to alter sAA values in PTSD patients. However, given its short half-life, it is not clear whether bedtime doses would have an effect on daytime sAA. In the present study, we assayed daytime sAA in 20 suicidal PTSD patients who were randomized to prazosin versus placebo at bedtime-only, and found no effect in daytime sAA. These findings are consistent with studies showing an advantage for twice daily dosing of prazosin in PTSD.

1. Introduction

Posttraumatic stress disorder (PTSD) is a serious psychiatric illness, often complicated by social disability and risk of suicide. Some of the symptoms of PTSD, such as enhanced startle reactions, hyper-vigilant scanning of the environment for threats, and nightmares are characteristic of the noradrenergic (NE) system 'fight or flight' response. Pathophysiologic studies of PTSD also support overactivity of the NE system [1].

A better understanding of the role of NE in PTSD would facilitate 'precision medicine', potentially identifying specific patients that would benefit from interventions that offset excess CNS NE tone. NE function could be measured by several potential techniques. An ideal technique would be an in vivo measurement of NE function in humans. However, in vivo measurement of NE function in humans is hampered by the lack of radioisotopes for positron emission testing imaging of the NE system. Outside of the CNS, the most direct measure of the NE system is neural traffic in an impaled sympathetic nerve [2]. While this measure has proven to be sensitive, it is highly technical, uncomfortable, and not suitable for clinical practice. Another less invasive measure of NE traffic is salivary α-amylase (sAA), which varies in proportion to NE activity [3].

A comparison of sAA in 10 adult, medication-free Bosnian War refugees with PTSD and 11 controls found higher sAA activity in the refugees, and the intensity of PTSD symptoms was positively correlated with sAA [4]. A different study recruited 18 adult PTSD sufferers (only 7 were receiving medications) and compared them with 20 trauma-exposed adults without PTSD and 20 controls, and found higher sAA activity in the PTSD sufferers [5].

As an antagonist of the α-1 NE receptor, prazosin would be expected to alter sAA values in PTSD patients. Indeed, a single dose of oral prazosin 3 mg leads to a doubling of sAA values within 3 hours after dosing in healthy controls [6]. The prazosin-mediated increase in sAA results from blockade of the α-1 NE receptor, and resulting unopposed action of NE on the β receptor. Activation of the β receptor leads to increases in sAA [7]. However, given its short half-life, it is not clear whether bedtime doses would have an effect on daytime sAA. Herein we report the results of a secondary hypothesis for a study whose principal aim was to examine the impact of a bedtime dose of prazosin on suicidality and sleep in suicidal PTSD patients [8]. For this secondary aim, we examined the effect of bedtime doses of prazosin on daytime sAA activity in PTSD patients, as a test of whether a night time intervention could be expected to have a beneficial carry over effect the next day [8].

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2. Materials and methods

The study was approved by the Augusta University (AU) Institutional Review Board (IRB), and carried out in accordance with the latest version of the Declaration of Helsinki. Participants were recruited through the outpatient psychiatry clinic at the Medical College of Georgia. Participants provided written, informed consent, and were paid $25.00 in compensation for their time. Prior to recruiting the first patient, the study trial design was registered at ClinicalTrials.gov and identified as NCT02199652.

A full description of the methods of the clinical trial can be found elsewhere [8]. Briefly, suicidal PTSD patients, who were already taking antidepressants or mood stabilizers, were randomized for 8 weeks of add-on therapy of prazosin versus placebo at bedtime. Weekly visits during the 8-week period of randomization allowed for weekly escalation of with prazosin doses as tolerated and weekly collection of salivary samples for sAA(Table 1).

2.1. Participants

Participants were enrolled if they met criteria for PTSD according to the Clinician Administered PTSD Scale (CAPS-5) [9], while other psychiatric diagnoses were made according to DSM-IV at the baseline visit [10]. Inclusion criteria included: age 18–65 years old, nightmare severity as measured by the Disturbing Dreams and Nightmare Severity Index (DDNSI) ≥ 10 [11], and at least moderate suicidal ideation intensity as measured with the Scale for Suicide Ideation (SSI) score ≥ 3 [12]. Co-morbid psychiatric diagnoses were permitted except for active substance abuse in the last 90 days, schizophrenia, or active mania. Patients with a clinical diagnosis of major neurocognitive disorder were excluded. Additional exclusion criteria were a history of fainting in the last 6 months, a history of hypotension, or blood pressure (BP) readings with systolic BP < 90 mm Hg, or diastolic <50 mm Hg.

Participants meeting criteria for comorbid major depressive disorder (MDD) were also required to receive sertraline ≥ 50 mg per day, or its equivalent, for ≥ 4 weeks before randomization. Participants meeting criteria for bipolar disorder, most recent episode depressed, received a mood stabilizing medication for ≥ 4 weeks before randomization. Participants were instructed to continue all other previously prescribed and ongoing psychotropics at stable doses throughout the study.

PTSD symptom severity was measured with the PTSD Checklist (PCL) [9, 13]. Insomnia severity was measured with the Insomnia Severity Index (ISI) [14]. Severity of depressive symptoms was measured with the Hamilton Rating Scale for Depression (24-item version) [15].

2.2. Randomized treatment

Randomized treatment lasted 8 weeks, with weekly clinic visits. The prazosin dosing schedule was adapted from Raskind [16], modified for only bedtime dosing (Table 1). Adherence to study medications was assessed at each visit with pill counts.

2.3. Salivary α-amylase assays

The participants did not eat a major meal within 60 minutes of saliva collection. Salivary samples were collected using oral swabs, allowing them to be completely saturated before removal. Samples visibly contaminated with blood were recollected. sAA values vary with the time of day, rising through the morning into early afternoon, before declining in late afternoon [17]. For this reason, strict attention was paid to collecting sAA samples at the same time each day, between noon and 5 PM. A sample was collected at each weekly visit for up to 8 weeks.

Each participant was instructed to place an oral swab between the lower lip and lower premolars for five minutes under supervision. After collection, samples were refrigerated within 30 minutes and then centrifuged within the next 4 hours. The samples were all centrifuged at 2000xg at 4 C for 10 minutes. After centrifugation, the samples were stored at $-20\ °C$ until analysis. Alpha-amylase (U/ml) activity was determined using the enzyme kinetic method as per the manufactured protocol (Sigma-Aldrich#MAK009). Briefly, to assay α-amylase activity, 50 μl of test sample/H2O were mixed with 50 μl amylase assay buffer and 50 μl α-amylase substrate mix and incubated at 25 °C for 3 min. After 3 minutes, the absorbance at 405 nanomole (nm) was measured to obtain optical density values for the initial time (T-initial). Samples were incubated at 25 °C and absorbance was measured at 405nm after every 5 minutes until the values of the samples (T-final) are near the highest standard (20 nm/well) provided. The enzyme activity was calculated by the following formula (Δ sample)*sample dilution factor/(reaction time) *(volume); Δ = absorbance test group T-final - absorbance test group T-initial. p-nitrophenol was used as positive control and activity was calculated using standard curve.

2.4. Statistical analysis

The correlation between first and last-observed sAA values was quantified using Pearson's r. The difference between the two time points was assessed using a paired t-test. A longitudinal mixed effects model was used to assess the change in sAA over time and to assess the effect of prazosin on these changes. 'Visit' was entered into the model as a class variable. Baseline means were constrained to be equal in the two groups. A compound symmetry structure was used to account for the within patient correlation over time. Linear contrasts were used to obtain estimates of the treatment effect at each time as well as the average treatment effect over all the times.

3. Results

Twenty patients were randomized, 10 to each treatment, and their demographic and clinical features are described in Table 2. Sixteen of the PTSD patients had comorbid major depression, while the other four patients met criteria for comorbid bipolar disorder, depressed phase. Seventeen patients were taking either a selective serotonin reuptake inhibitor (SSRI) or a serotonin-norepinephrine reuptake inhibitor (SNRI).

Table 1
Prazosin bedtime dose titration schedule.

Week/Day	Men's Dose (mg/day)	Women's Dose (mg/day)
Days 1–2	1	1
Days 3–7	2	2
Week 2	4	2
Week 3	6	4
Week 4	10	6
Week 5	15	10
Week 6	20	10

Table 2
Demographic and Clinical Features of Participants. Mean $\pm$ SD unless otherwise specified.

	Placebo (N = 10)	Prazosin (N = 10)	Total (N = 20)
Age (years)	43.2 ± 12.7	36.3 ± 15.9	39.8 ± 14.5
Gender (% F)	80	90	85
Race (% W)	60	80	70
MDD (%)	80	80	80
Bipolar depression (%)	20	20	20
GAD (%)	60	70	65
OCD (%)	10	0	5
CAPS-5 total	44.4 ± 5.3	47.9 ± 10.9	46.1 ± 8.4
PCL total	62.4 ± 8.0	60.8 ± 11.7	61.6 ± 9.8
PCL minus sleep	58.9 ± 7.5	56.9 ± 10.9	57.9 ± 9.2
DDNSI	20.9 ± 4.3	23.4 ± 5.9	22.2 ± 5.2
ISI	18.7 ± 3.6	22.0 ± 4.2	20.4 ± 4.2
HRSD	28.5 ± 6.0	26.4 ± 6.6	27.5 ± 6.2
SSI	14.8 ± 7.3	13.7 ± 6.9	14.3 ± 7.0

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Three of the four bipolar patients took neither a SSRI nor a SNRI, but instead took lamotrigine (N = 2) or risperidone (N = 1). Six patients took benzodiazepines, and six were using trazodone. The final highest prazosin dose for men and women combined was 5.5 ± 3.5 mg for prazosin and 7.6 ± 5.3 mg for placebo, with no significant mean differences in dose between treatment groups (p = 0.31).

Ninety-six sAA samples were collected from the 20 subjects. Of these, baseline values for sAA were available for 8 of the placebo patients and 9 of the prazosin patients, with values of 3922.2 ± 497.3 and 3552.2 ± 1466.0 for the placebo and prazosin groups, respectively (p = n.s.). Overall, first and last-observed sAA values were moderately correlated (Pearson's r = 0.60, p < 0.05), and there was no significant difference between the first and last-observed values (p = 0.99). There was no overall change in sAA over time (p = 0.89). Additionally, there was no effect of prazosin on sAA, with an average treatment effect of -15.7 ± 374.8 (95% confidence interval $-765.1, 733.7$).

4. Discussion

sAA values were stable across time for the combined treatment groups. While bedtime-doses of prazosin had a statistically significant effect on nighttime variables such as nightmares, insomnia, and depression scores (which include sleep items) [8], there was no signal of a carry-over effect of bedtime-only dosing of prazosin on next-day sAA activity. This is consistent with our clinical report showing no effect of bed-time only dosing of prazosin on daytime PTSD symptoms [8], as the short half-life of prazosin would not be expected to be present in blood in appreciable quantities 12 hours after bedtime dosing, due to its short half-life.

This study has a series of limitations. First, the small sample size could lead to a Type II error. Second, we only collected sAA sample in the afternoon, and it is possible that a collection in the morning, closer in time to the prior night's bedtime dosing, could have shown an effect. Third, sAA values are influenced by parasympathetic tone, in addition to sympathetic tone. Indeed, blockade of α-2 receptors can lead to strong parasympathetic effects and corresponding change in sAA [18], but such effects have not been described yet with prazosin. A more complete assessment of the effects of prazosin on sAA should include consideration of parasympathetic influences.

5. Conclusions

In conclusion, our results suggest that a single bedtime dose of prazosin is inefficient in influencing daytime measures of sAA, as an indicator of daytime NE physiology of PTSD patients. Increasing the bedtime dose beyond our present dosing schedule is impractical because of increasing risk of serious side effects such as hypotension. In contrast, multiple daily doses of prazosin may be more likely to affect daytime sAA, as a previous study has shown superior control of symptoms with twice daily dosing of prazosin in PTSD patients [19].

Declarations

Author contribution statement

William Vaughn McCall: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Anilkumar Pillai, Chirayu D. Pandya, Laryssa McCloud, Jason A. Moraczewski, Liniya Tauhidul, Nagy A. Youssef, Doug Case, Peter B. Rosenquist: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare the following conflict of interests:

William Vaughn McCall: Research support – Merck, MECTA Corp; Royalties – Wolters Kluwer Publishing; Speakers fees – CME Outfitters.

Peter B. Rosenquist: Research support – MECTA, GlaxoSmithKline and Lumosity.

Nagy A. Youssef: Research support – Merck, MECTA.

The remaining authors declare no conflict of interest.

Additional information

The clinical trial described in this paper was registered at Clinical Trials.gov under the registration number NCT02199652.

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