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Effects of Gabapentin Enacarbil on Cortical Arousals, Heart Rate, Blood Pressure and Anterior Tibialis EMG Responses Associated with PLMs in Restless Legs Syndrome

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Abstract

Objective: This study was conducted to investigate the effects of gabapentin enacarbil (GEn) on sleep EEG, heart rate (HR), blood presssure, anterior tibialis EMG activity (PLMs power) and subjective complaints in subjects with moderate to severe RLS and disturbed sleep.

Methods: This was a single site, single-blind, placebo run-in, fixed dose single group polysomnography (PSG) study. Eligible subjects (age 24-66 years) were treated with placebo for one week and GEn (600 mg/day) for 4 weeks. Two in-laboratory PSGs were collected for adaptation and baseline at the end of the placebo run-in period and for re-adaptation and efficacy assessment at the end of the 4-week treatment period. The primary endpoint was the difference in PSG derived cortical arousal intensity (arousal scale, 0-9) associated with PLMs between 4 weeks of treatment with GEn and placebo. Secondary endpoints included changes in HR responses (ΔHR), nocturnal systolic blood pressure (SBP) changes (>10 mmHg) secondary to PLMs and PLMs power. Other PSG and subjective measures were assessed.

Results: Of 20 subjects enrolled, 18 completed the study. Subjects treated with GEn did not show significant improvements in cortical arousal intensity and ΔHR. However, subjects showed reduced PLMs power (p= 0.013) and associated reductions in nocturnal SBP per hour of sleep (p= 0.041) GEn showed significant improvement in other PSG parameters and subjective endpoints.

Conclusion: The data suggests that GEn reduces the frequency and power of PLMs and the corresponding SBP changes in subjects with RLS. Despite reducing the total number of PLM associated arousals and nocturnal HR, the study did not demonstrate consistent effects of GEn on cortical arousal intensity and corresponding HR changes associated with PLMs.

Clinical Trial Registration**:** ClinicalTrials.gov identifier: NCT02424695

Keywords: RLS, GEn, PLM power, Arousals Intensity, Heart Rate, Blood Pressure, Polysomnography

Abbreviations

AASM: american academy of sleep medicine
AE: adverse event
AI: arousal index
ALT: alanine aminotransferase
AST: aspartate transaminase
BDI -II: beck depression inventory
BP: blood pressure
BTBD9: bric-a-brac domain containing 9
CPAP: continuous positive airway pressure
EEG: electroencephalogram
EMG: electromyogram
GEn: gabapentin enacarbil
HCG: human chorionic gonadotrophin
HR: heart rate
HUTT: head up tilt table test
IRB: institutional review board
IRLS: international restless legs scale
IRLSSG: international restless legs syndrome study group
LOCF: last-observation-carried-forward
LPS: latency to persistent sleep
PGIC: patient global impression of change
PLM: periodic limb movements
PLMAI: periodic limb movements associated with arousal
PLMI: periodic limb movements per hour of sleep
PSG: polysomnography
PSQ-RLS: post- sleep questionnaire for RLS
PTT: pulse transit time
REM: rapid eye movement sleep
RLS: restless leg syndrome
SAE: serious adverse event
SBP: systolic blood pressure
SE: sleep efficiency
SSQ: subjective sleep questionnaire
SWS: slow wave sleep
TST: total sleep time
ULN: upper limit normal
WASO: wake time after sleep onset

Introduction

Restless Leg Syndrome (RLS) is most linked to symptoms manifesting during wakefulness, however, the majority of RLS patients also suffer from periodic limb movements during sleep (PLMs). These are spontaneously and rhythmically occurring kick -like movements of the limbs that maybe occur as often as every twenty to forty seconds during sleep. As such, PLMs are often associated with EEG arousals and awakenings thought to contribute to a sense of poor sleep quality [1,2]. In addition, PLMs are accompanied by increases in heart rate and changes in cerebral activity in terms of increases in the power of alpha, delta and theta EEG frequencies [3]. These PLM related EEG changes occur even when PLMs do not result in clear arousals [4]. Interestingly, even though PLMs can occur in patients without RLS, the frequent occurrence of PLMs with RLS suggest a common etiology. In support of this, Winkelman showed a common gene variant in BTBD9 in patients with PLMs and without RLS [5].

Whereas the precise clinical significance of PLMs is not well understood, it is thought that factors contributing to frequent and chronic disruptions in sleep with resultant transient heart rate and blood pressure elevations may, over time, increase the risks of developing heart disease [6,7]. This is supported by data from the Sleep Heart Health Study which suggests that the frequent and chronic arousals from sleep in RLS patients with PLMs are associated with greater incidence of heart disease and stroke [8].

The degree to which frequent and chronic disruptions in sleep pose an increased risk factor in these patients is not well understood. However, given that physician diagnosed RLS was reported in 2.2% of over 2800 senior men, with accompanying PLMs occurring at a rate of 15 per hour of sleep in almost 60% of the patients, the scope of the problem is not insignificant. The data suggests that PLM mitigation may have long term positive effects on cardiovascular health [9].

Technical advances in quantifying arousals in the sleep EEG have led to an appreciation that individual PLMs and nocturnal arousals differ widely in their intensity. Utilizing an automatic scoring system for scaling arousal intensity on a nine-point scale, Azarbarzin and colleagues demonstrated that PLM associated increases in heart rate were highly correlated with arousal intensity [10]. Further, they found a strong correlation between PLM intensity defined as the maximum amplitude of anterior tibialis EMG activity during PLMs and their arousal scale [11].

We previously demonstrated that the power of PLMs in RLS may be a more useful parameter in predicting blood pressure changes than the commonly used parameter of PLM frequency or frequency of PLM arousals, extending the findings of Azarbarzin by demonstrating an additional relationship between blood pressure and PLM power [12]. Additionally, we reported that the intensity of arousals associated with PLMs was a better predictor of subjective sleep complaints in RLS patients than frequency [13].

While gabapentin has been marketed as an anticonvulsant and analgesic, gabapentin enacarbil (GEn) was approved by the Food and Drug Administration exclusively for the indications of treatment for moderate to severe RLS in adults (600 mg daily) and postherpetic neuralgia (600 mg twice daily) [14]. GEn has been shown to be effective in decreasing the discomfort of RLS syndrome as well as the number of PLMs and PLM arousals that often concomitantly occur during the night.[15] Studies assessing the efficacy of GEn have similarly focused on overall RLS symptoms without assessing its impact on PLM power or the cardiac response to these nightly disruptions. The purpose of the current study was to evaluate and compare the impact of four weeks of nightly GEn use on cortical arousal intensity, heart rate and blood pressure associated with PLMs and PLM power in patients with RLS.

Materials and Methods

Study Design

This was a phase IV single center, single-blind, placebo run-in, fixed dose single-group polysomnography study to assess objective and subjective effects of GEn on sleep EEG, heart rate, blood pressure, and anterior tibialis EMG responsivity in patients with

RLS. The study was conducted in compliance with the declaration of Helsinki and International Conference on Harmonization guidelines. The protocol and informed consent were reviewed and approved by an independent institutional review board (IRB). Written informed consent was obtained from all the subjects before any study related procedures were initiated.

The total duration of the study from the first subject enrolled to the last subject completed was approximately 18 months. The study comprised 8 visits over a period of up to 8 weeks for eligible subjects including a 1 to 3-week Screening/Washout Period, a 1-week Placebo Run-in Period, and a 4-week Treatment Period with GEn 600 mg (Figure 1). The first placebo was administered within 1 to 3 weeks after Screening/Washout. Subjects were required to satisfy subjective and objective sleep disturbance criteria to qualify to enter the treatment phase.

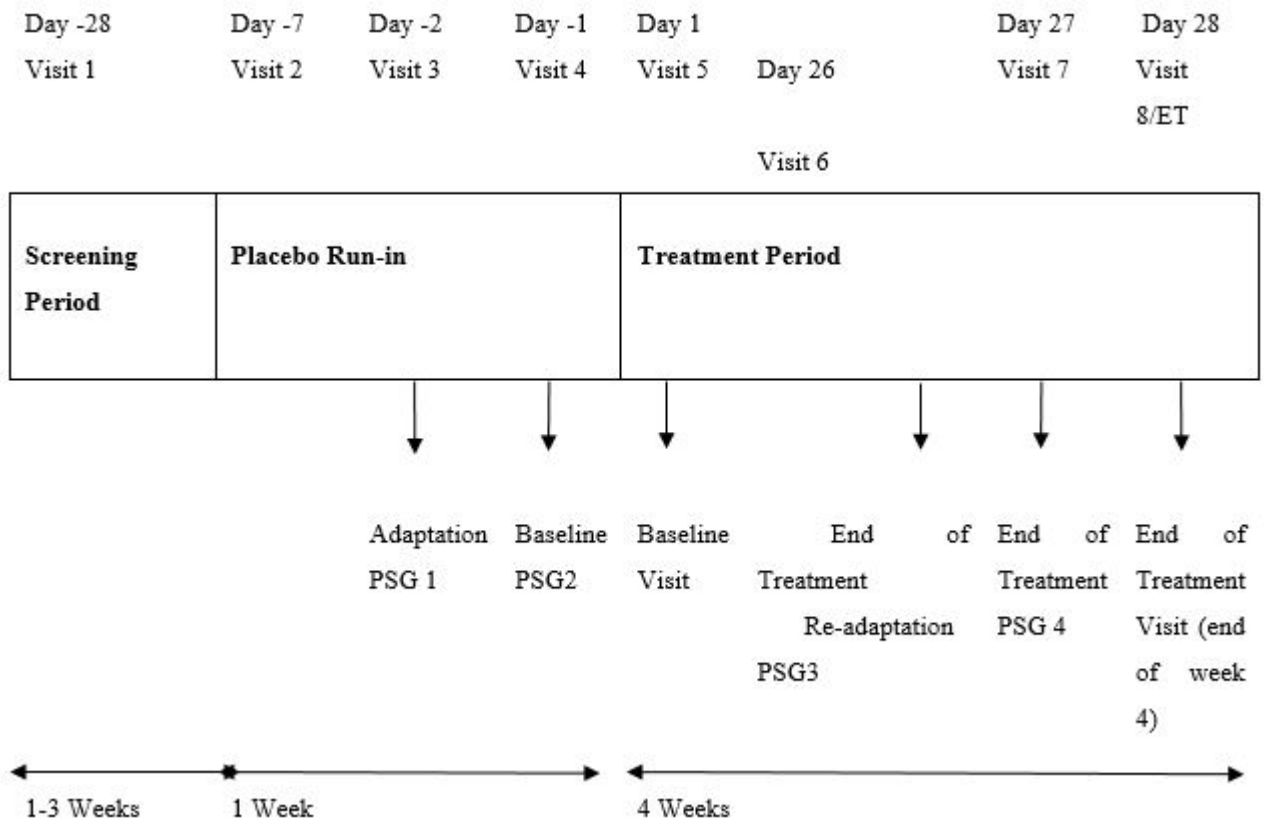

Figure 1: Schematic overview of study design

Treatment Schedule

During this study, subjects were dosed with placebo for one week (single blind run-in) followed by GEn 600 mg once daily for 4 weeks. Subjects were blinded to sequence allocation. Study drug was supplied as masked tablets of GEn 600 mg and matching placebo. Subjects were instructed to take their study medication once daily with food in the evening at approximately 5 PM. Each tablet was to be swallowed whole and not divided, crushed, or chewed.

Concomitant Medications

Subjects could continue stable doses/ regimens of medications that in the opinion of the investigator would not interfere with the conduct of the study. Pain killers such as acetaminophen, aspirin and other non-steroidal anti-inflammatory medications were allowed. Treatments with any drug therapy that could affect RLS efficacy assessments were prohibited. This included levodopa/carbidopa, dopamine agonists (e.g., pramipexole, ropinirole), pregabalin or gabapentin and were to be discontinued at least 3 weeks prior to V2.

Similarly, sedative compounds including benzodiazepines and sleep aids, opioids, antipsychotics, tricyclic antidepressants, tramadol, mood stabilizers were to be discontinued at least 2 weeks prior to V2.

Study Population and Procedures

After each subject signed the IRB approved informed consent form, a detailed medical and RLS history, previous and current treatments for RLS, history of augmentation with treatment, history of concomitant medications and evidence of sleep disturbance were obtained. Severity of RLS was determined using the IRLS scale. The Berlin questionnaire [16] was used to identify those subjects at high risk for obstructive sleep apnea. Other assessments included a physical and neurological exam, an electrocardiogram and depression levels (Beck depression inventory/BDI-II) [17]. Subjects were given a sleep diary with instructions to record their daily sleep times, as well as a numeric rating for subjective sleep quality. After the review of the clinical laboratory results, the subjects were instructed to wash out of any prohibited medications. Sleep diaries were reviewed to ensure that subjective sleep difficulty measurements met the entry criteria for the run-in period along with RLS symptoms frequency and IRLS scores. Two PSGs, one for adaptation and the second for baseline establishment, were obtained at the end of the run-in period and used for inclusion/exclusion. Subjects were instructed to report to the sleep laboratory 1 hour before their usual bedtime for all PSG visits. The results of the screening visit assessments, placebo-initiation visit, sleep diary, first 2 PSGs, and inclusion and exclusion criteria were thoroughly reviewed before the subjects were enrolled into the study. Baseline subjective assessments were carried out on the morning of the second PSG (V5). Two additional PSGs, one for re-adaptation and the second for GEn efficacy were obtained at the end of the 4-week treatment period along with subjective assessments on the morning of the last PSG.

Inclusion Criteria

Male or female subjects ages 18 -75 years meeting the International Restless Legs Syndrome Study Group (IRLSSG) diagnostic criteria for primary moderate to severe RLS at screening along with clinically significant sleep disturbance were selected for this study. Eligible subjects had a minimum six-month history of RLS with symptoms manifesting on at least 15 days during the month prior to screening (or if on treatment, similar symptom frequency prior to initiating treatment) and a history of sleep disturbance due to RLS over the last 3 months. RLS symptoms must additionally have occurred on at least four of the seven consecutive days/evenings in the week prior to V2. Subjects must also have had an IRLS score of ≥15 points (at visits 1 and 2) and a score of ≥2 on item 4 indicating moderate to severe sleep disturbance. Disturbed sleep was defined as a subjective history of wake after sleep onset (WASO) of at least 45 minutes and total sleep time (TST) of less than 6.5 hours. These levels of sleep difficulty must have been occurring on at least three nights per week within the three months prior to study entry and at least three nights out of seven on the Subjective Sleep Questionnaire (SSQ) during the week prior to V2. Screening continued through the placebo run in period and the first two PSG nights (visits 3 and 4) with inclusion requiring an objective WASO of at least thirty minutes, a TST of less than seven hours and a periodic limb movement index (PLMI) of at least ten per hour for the first two PSGs. The subjects understood and were willing to cooperate with the study procedures before they signed the informed consent form. They were instructed to maintain a normal daytime awake and nighttime sleep schedule.

Exclusion Criteria

Subjects presenting with unstable, or uncontrollable medical or psychiatric conditions were excluded from further study participation. Subjects showing obstructive sleep apnea with an apnea- hypopnea index of 15 or more episodes per hour of sleep during the first 2 PSGs were excluded. However, subjects with a history of obstructive sleep apnea controlled with nasal continuous positive airway pressure (CPAP) with demonstrated nightly compliance could participate in the study. Subjects with history of depression were excluded if the BDI-II showed moderate to severe depression or were deemed to be at significant risk for suicide. Other exclusion criteria included subjects with clinically significant laboratory abnormalities indicating hepatic impairment (ALT or AST > 2x ULN or alkaline phosphatase or bilirubin >1.5xULN), impaired renal function (estimated creatinine clearance <60 ml/min),

serum ferritin level <20 ng/ml in addition to clinically significant ECG abnormalities, uncontrolled hypertension, the presence of additional sleep disorders or evidence of secondary RLS, neurologic disease or movement disorders, moderate to severe fibromyalgia, moderate to severe rheumatoid arthritis, history of epilepsy or seizure disorders, active hepatitis B or C. In addition, subjects were excluded if they were unable to discontinue prohibited medications, had excessive caffeine use (defined as a daily consumption of more than 600 mg of caffeine or other xanthines over the month prior to screening) or were unwilling to refrain from consuming any caffeinated food or beverages within 8 hours prior to any PSG assessments, or alcohol use >14 units/week or more than 5 alcoholic units in any single day over the month preceding the screening visit or were unwilling to refrain from consuming alcohol within 24 hours of any PSG assessment, were working shift work, currently pregnant or lactating, seen as non-compliant, and history of substance abuse within 1 year prior to the study. Treatment history exclusions included history of allergy to GEn, or augmentation or early morning rebound of RLS symptoms without a positive response to GEn treatment.

Efficacy Assessments

Polysomnography Assessment

The total PSG recording time was 8 hours per session and were obtained using SOMNOscreen Plus PSG system (Somnomedics, Randersacker, Germany) at baseline (visits 3 and 4) and at the end of the 4-week treatment period (visits 6 and 7). All PSGs were carried out to match the subjects' usual bedtime based on sleep diaries. The PSG scoring was performed blindly in accordance with AASM criteria [18]. Scoring of standard PSG parameters was carried out by a single master scorer. The second nights of recordings at Baseline (V4) and at the end of the Treatment Period (V7) were included in efficacy analyses. Scaling of cortical arousals associated with PLMs and corresponding heart rate changes were conducted by Research and Development Lab (YRT limited) using Azarbazin algorithm.

Primary Objective Endpoint: The primary endpoint was the mean change from baseline to week four in PSG-recorded intensity of cortical arousals associated with PLMS. Intensities of cortical arousals were scaled using the method described by Azarbarzin et al [10]. Arousals were initially scored in sleep and subjectively assigned a value from 0-9, with 9 being the most intense arousals. The arousal intensity was then quantified using Wavelet Transform analysis of C3/A2 and C4/A1 EEG signals.

Secondary Objective Endpoints: The secondary objective endpoints included: mean change from baseline to the end of the treatment period in heart rate (ΔHR) associated with arousals secondary to PLMs, mean change from baseline to end of treatment period in PLM power and associated blood pressure changes, and mean change from baseline in PSG sleep parameters. Heart rate changes associated with cortical arousals secondary to PLMs were measured by identifying the highest HR in the interval 2-12 seconds preceding each arousal (baseline) and comparing it to the highest HR during the eight second period after the arousal. The difference from baseline represents the change in heart rate associated with the arousal [10].

The power of PLMs (PLMs average power in decibels) with or without associated arousals were derived using the Somnoscreen Plus algorithm in which PLMs power is expressed as: PLMs average amplitude [dBs]/PLMs average duration, where the average amplitude is the relative power of the PLM event (related to the time) in comparison to the lowest value in the complete 8-hour recording (background noise). One decibel equals 10 times the common logarithm of the power ratio.

Systolic blood pressure (SBP) changes associated with PLMs were also derived using the Somnoscreen Plus algorithm measuring blood pressure via the pulse transit time (PTT) [19,20]. The PTT is calculated as the interval between R-wave on ECG and the arrival of the corresponding pulse wave (determined from finger plethysmography signal) at the fingertip. A single initial BP measurement used for device calibration was obtained while subjects were resting in bed for at least 5 minutes and prior to lights off. Any change in SBP of over 10 mmHg within 3-30 seconds after a PLM event reflected the change in blood pressure associated with PLMs. The PLM systolic index was derived as the number of SBP increases correlating to PLM events per hour of sleep. PLM systolic (%) values were derived as percentages of SBP increases correlating to PLM events. Only records with low artifact were used for analysis.

Additional secondary PSG parameters included total sleep time (TST), wake after sleep onset (WASO), latency to persistent sleep onset (LPS), defined as time from lights out to the first consecutive 2 minutes of uninterrupted sleep, sleep efficiency (SE), arousal index (AI), defined as number of arousals per hour from sleep onset to lights on, sleep stage percentages (%TST), PLMI (periodic limb movements per hour), PLMAI (periodic limb movements associated with arousal per hour).

Subject Reported Assessments

Secondary subjective efficacy endpoints reported in this paper: included the following subject reported assessments: International RLS (IRLS) Rating Scale Study scores, Proportion of responders ("moderately better" to "a great deal better") on the Patient Global Impression of Change (PGIC) scale [21] and the Post Sleep Questionnaire (PSQ-RLS). The PSQ was administered at baseline (V5) and end of treatment period (V8) while the IRLS Rating Scale was administered at the screening visit (V1), placebo initiation visit (V2) and V5 and V8.

IRLS Rating Scale: This validated 10-item scale was developed by the International Restless Legs Syndrome Study Group (IRLSSG) to assess the severity of RLS symptoms. Subjects are asked the questions and rate their symptoms in the past week from 0 to 4, therefore leading to a maximum total score of 40. A score of 11 to 20 is indicative of moderate RLS [22].

Post Sleep Questionnaire: This self-report instrument was developed to evaluate sleep disturbance in subjects with primary RLS over the past weeks using 4 Likert-type scale questions and 1 quantitative, open-ended question. It assesses several single-item sleep domains, including overall sleep quality, overall daytime functioning, frequency of nighttime RLS symptoms, and RLS-related sleep disturbances and latency over a one-month time interval. The score ranges from 2 to 23 with higher PSQ scores indicating worse sleep (greater sleep difficulties) [23].

Safety Assessments

Any abnormal findings such as abnormal laboratory results, clinically significant symptoms, physical/neurological examination findings, hypersensitivity to GEn or any other medications, worsening of the underlying disease, emergence and worsening of depression, drug overdose/dependency/misuse, and/or pregnancy were considered as adverse events (AEs).

Vital signs were obtained at every visit to identify changes in heart rate and blood pressure. Electrocardiogram was performed at Screening and physical exam at placebo initiation visit. Urine Pregnancy test using HCG-dip stick was performed at Screening, Baseline and End of study visits. Laboratory assessments (hematology and clinical chemistry) were done at Screening. The BDI-II was administered at the screening visit to identify and assess severity of current symptoms of depressive disorders. All subjects receiving at least one dose of study medication or matching placebo were monitored for safety. The treatment emergent adverse events, subject-reported or observed by the investigator, were assessed for severity and causality.

Statistical Analysis

The primary efficacy endpoint was the reduction from placebo-baseline in cortical arousal intensity associated with PLMs after treatment with GEn 600 mg. A sample size of 20 subjects was estimated by a priori power analysis to achieve 92% power for a 2-sided 5% paired *t*-test with an effect size of 0.80. Twenty subjects enrolled in the study with only 18 completers. The primary efficacy analysis was performed on the per-protocol population who completed the study taking 600 mg/day of the study drug.

All data were analyzed using IBM® SPSS® Statistics 26. Polysomnography and secondary subjective end points were each analyzed

using a paired *t*-test. Only the 95% confidence intervals (95% CIs) for the true mean treatment difference are reported for primary and secondary efficacy endpoints.

All enrolled subjects who received one or more doses of the study and placebo treatment were included in safety assessments. Safety and tolerability analyses focused primarily on frequency of adverse events and the changes in vital signs.

Results

Subject Disposition and Characteristics

A total of 81 subjects were screened, of whom 14 (17.3%) took only placebo treatment (placebo run-in failure) and 20 (24.7%) received placebo and GEn treatment. During the study, treatment with GEn was discontinued in 2 subjects due to non-compliance and an AE unrelated to study drug (Figure 2). In total, 18

subjects were included in the per-protocol analysis for the assessment of primary and secondary polysomnography endpoints and

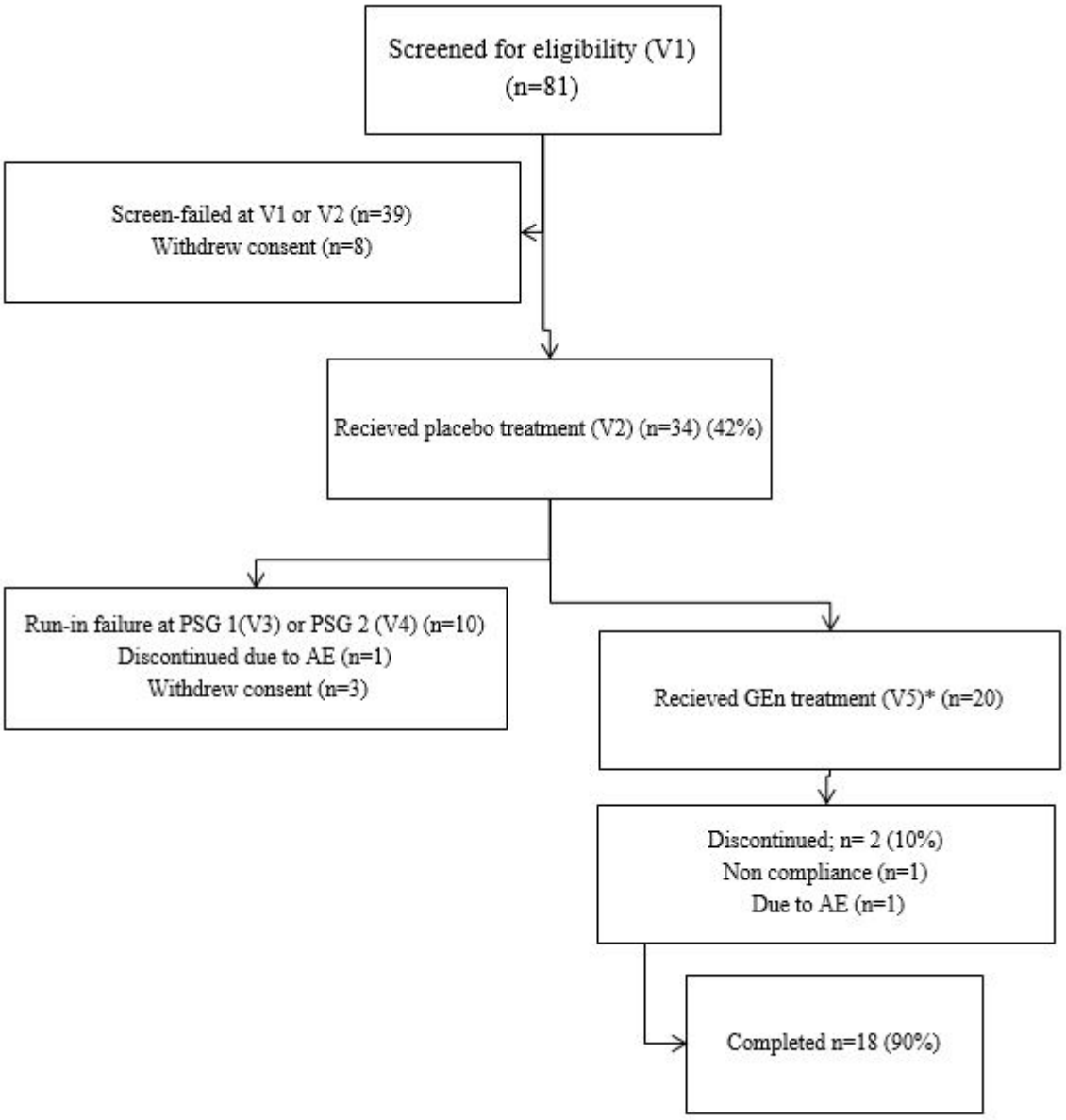

Figure 2: Flow of subjects through the study

secondary subjective endpoints. Subjects were predominately women (15 [75%] of 20), white (16 [80%] of 20) with a mean age of 54.0 years (range 24–66 years) and a mean weight of 196.7 lbs. (n= 20, ± 54.0). Ten percent of the subjects were Hispanic. The mean duration of RLS was 17.2 years (n=20, ± 14.2) and the mean years since diagnosis of RLS was 10.9 (n=20, ± 11.2).

Study subjects showed sleep disturbance at baseline as shown by PSG parameters for sleep continuity and architecture. Subjects averaged 38 ±27.3 PLMs/hr (9.8-101.9) and a PLMAI of 7.8±8.9 (1.0-34.0). Mean PLMs power was 15.7±3.8 dBs (11.5-28.5). The mean arousal scale values were relatively low averaging 3.6± 0.9 (n=15, 2.31-5.78) with a median of 3.3and a mode of 2.31. The corresponding ΔHR averaged 9.3± 3.5 (n=15, 2.67-15.65) with a median of 8.5 and a mode of 2.67. The mean PLM systolic index was 7.7±10.8 (n=14, 0.2-42.9) and the mean PLM Systolic (%) was 5.21±4.1 (0.2-11.5).

Subjects also showed clinically relevant sleep disturbance in the PSQ-RLS total score. The Average total IRLS score was 17.2 (n=18, ± 4.5) consistent with reported averages for moderate / severe RLS severity.

Efficacy Analyses

Polysomnography Assessments

GEn administration did not result in significant improvements in arousal scale and associated ΔHR. However, GEn reduced the average HR during the night (end of treatment paired difference: -3.3, t_{14} [p-value] = -2.592 [0.021]). The arousal index, PLM index, PLMAI and PLM power were reduced with GEn treatment versus placebo (end of treatment paired difference: -4.5 events/h, t_{17} [p-value] = -2.183 [0.043], -10.7 events/h, t_{17} [p-value] = -2.533 [0.021], -2.9 events/h, t_{17} [p-value] = -2.740 [0.014] and -1.4 dBs, t_{17} [p-value] =-2.758 [0.013]). GEn also significantly reduced the PLM systolic index, PLM systolic (%) and SBP during night: (end of treatment paired difference: -4.3events/h, t_{13} [p-value] = -2.268 [0.041], -7.1, t_{13} [p-value] = -2.333 [0.036] and -9.3 mmHg, t_{13} [p-value] = -2.373 [0.034]). Subjects treated with GEn showed significant improvements in PSG-determined TST, WASO and sleep efficiency versus treatment with placebo: (end of treatment paired difference: 23.6 minutes, t_{17} [p-value] =2.145 [0.047], -20.1 minutes, t_{17} [p-value)] = -2.284 [0.036] and 5.1%, t_{17} [p-value] =2.692 [0.015]. However, LPS and sleep stages were not affected by GEn (Table 1).

Secondary Subjective Efficacy Endpoints

Sixty one percent of the subjects were considered responders to GEn ('moderately better "to a great deal better') on the Patient Global Impression of Change (PGIC). Treatment with GEn resulted in statistically significant differences from placebo in IRLS total scores and PSQ (t_{17}(p-value) = -4.406 (0.000), t_{17} (p-value) = -4.015 (0.001)) (Table 2).

Table 1: PSG parameters of RLS subjects at placebo-baseline and after 4-week treatment with GEn

	GEn, Mean (SE)	Placebo, Mean (SE)	Paired difference (95% CI)	*t_{17} (p-value)
TST, min	394.4 (9.6)	370.8 (8.3)	23.6 (0.4, 46.9)	2.145 (0.047) ***
WASO, min	70.1 (9.5)	90.2 (6.7)	-20.1 (-38.6, -1.5)	-2.284 (0.036) ***
Sleep efficiency %	83.2 (1.9)	78.1 (1.5)	5.1 (1.1, 9.1)	2.692 (0.015) ***
LPS, min	19.6 (3.8)	21.6 (2.9)	-2.0 (-11.3, 7.4)	-0.442 (0.664)
Sleep stage %				
REM, % of TST	15.8(1.5)	15.9 (1.5)	-0.1 (-3.4, 44.5)	-0.223 (0.826)
1, % of TST	7.6 (1.1)	8.8 (0.8)	-1.2 (-2.4, 0.1)	-1.908 (0.073)
2, % of TST	53.0 (2.8)	50.7 (2.6)	2.2 (-2.7, 7.1)	0.965 (0.348)
SWS, % of TST	14.6 (2.5)	13.2 (2.0)	1.4 (-2.6, 5.4)	0.745 (0.467)
Arousal index, events/h	22.4 (3.3)	26.9 (2.6)	-4.5 (-8.8, -0.1)	-2.183 (0.043) ***
PLMs index, events/h	27.4 (5.7)	38.1 (6.4)	-10.7 (-19.6, -1.8)	-2.533 (0.021) ***
PLMAI, events/h	4.8 (1.9)	7.8 (2.1)	-2.9 (-5.2, -0.7)	-2.740 (0.014) ***
PLM power, dBs	14.2 (0.6)	15.7 (0.9)	-1.4 (-2.5, -0.3)	-2.758 (0.013) ***
Arousal Scale (n=15) **	3.7 (0.2)	3.5 (0.2)	0.2 (-0.1, 0.6)	1.269 (0.225)
ΔHR (n=15) **	8.7 (0.9)	8.9 (0.8)	-0.2 (-1.3, 0.9)	-0.452 (0.658)
HR during night (n=15) **	65.2 (2.2)	68.5 (2.4)	-3.3 (-6.1, -0.6)	-2.592(0.021) ***
SBP during night (mmHg) (n=14)**	115.6 (3.8)	124.9 (5.2)	-9.3 (-17.7, -0.8)	-2.373 (0.034)
PLM Systolic index, events/h (n=14) **	3.4 (1.1)	7.7 (2.9)	-4.3 (-8.5, -0.2)	-2.268 (0.041) ***
PLM Systolic (%) (n=14) **	19.5 (3.0)	26.6 (4.2)	-7.1 (-13.8, -0.5)	-2.333 (0.036) ***

*t and p values are based on paired t-test comparison between GEn and placebo-baseline. Analyses were performed on the per-protocol population (n=18) that completed the study taking 600 mg/day of GEn. ** Sleep recordings with significant artifact were excluded from the analysis resulting in a smaller sample size. ***Significant at 0.05 level. 95% CI, 95% confidence interval of the difference, TST, total sleep time, WASO, wake after sleep onset, LPS, latency to persistent sleep defined as time from lights out to the first consecutive 2 min of uninterrupted sleep, REM, rapid eye movement, SWS, slow wave sleep (stage 3 sleep), PLMs index, periodic leg movements per hour of sleep, PLMAI, periodic leg movements arousals per hour of sleep, Arousal scale, cortical arousal intensity associated with PLMs ranging from 0 to 9, ΔHR, change in heart rate associated with arousals secondary to PLMs, HR, heart rate, SBP, nighttime systolic blood pressure, PLM systolic index, the number of SBP increases correlating to PLM events per hour of sleep, PLM systolic (%), percentage of SBP increases correlating to PLM events, SE, standard error.

Table 2: Subjective parameters of RLS subjects at placebo-baseline and after 4-week treatment with GEn

	GEn, Mean (SE)	Placebo, Mean (SE)	Paired difference (95% CI)	*t_{17} (p-value)
IRLS total score		25.2 (1.1)	-7.9 (-11.7, -4.1)	-4.406 (0.001) **
PSQ-RLS total score	17.2 (1.6)	18.7 (0.4)	-5.1 (-7.8, -2.4)	-4.015 (0.001) **

**t* and p values are based on paired *t*-test comparison between GEn and placebo-baseline. Analyses were performed on the per-protocol population (n=18) that completed the study taking 600 mg/day of GEn. **Significant at 0.05 level. 95% CI, 95% confidence interval of the difference, IRLS, international restless legs scale, PSQ-RLS, post sleep questionnaire

Safety Assessments

No drug related AEs or SAEs were observed in the study. One subject experienced a concussion due to a fall from a sport activity and was discontinued during the placebo run-in period. Another subject was withdrawn during the treatment period after it was discovered that she had a previously denied history of bipolar depression that worsened because of withdrawal from a previously unreported antidepressant. No clinically significant weight or blood pressure changes were observed.

Discussion

In this study, we found that GEn significantly reduced the EMG power and total number of PLMs in patients with RLS. We also found GEn reduced the number of PLMs with arousals, total number of arousals (with and without PLMs) as well as improvements in WASO and total sleep time. Concomitantly, GEn significantly blunted systolic blood pressure increases associated with PLMs with and without arousals. GEn also resulted in improvements in subjective reports in sleep quality and daytime functioning. However, we did not find the expected significant changes in cortical arousal intensity and heart rate associated with PLMs with GEn treatment.

PLMs are frequently accompanied by EEG arousals or awakenings resulting in morning complaints of fatigue or non-restorative sleep. The onset of arousals occurs before initiation of each PLM suggesting they are not a consequence of the limb movement but instead reflect an underlying disorder of arousal [24]. Ferrillo et al reported that the onset of PLMs is preceded by increases in Delta power and heart rate and followed by activation of higher EEG frequencies. Thus, PLMs often begin with a (protective) increase in Delta power followed by activation of higher frequencies leading to arousal [25]. Despite GEn's dampening effect on EEG activation, we did not observe consistent predictable changes in arousal intensity or heart rate associated with PLMs of varying power. This data may suggest an association between PLM power and cardiovascular variables that is independent of cortical EEG arousal intensity. It is likely that the soporific and depressant effects of GEn rather than direct effects were sufficient to raise arousal thresholds to minimize sleep disruption resulting in decrease in number of arousals. Our failure to detect reduction in arousal scale may also be due to the low average intensity and low variability among the arousal intensity scale values seen in this study. Our failure to detect reduction in heart rate in association with PLMs is likely in part due to methodologic issues especially since the overall nighttime heart rate and PLM induced systolic blood pressure increases were significantly blunted with GEn treatment. In fact, heart rate and blood pressure increases can be seen even in PLMs not resulting in arousals. PLM related increases in heart rate have been reported in studies measuring heart rate over various and inconsistent number of seconds prior to and after each PLM [26,27]. As such, whereas we observed the expected changes by measuring heart rate 12 seconds before and 8 seconds after an event, heart rate reductions with GEn did not reach significance. In addition, back-to -back PLMs were observed in several patients, with the second PLM occurring before the prior PLM could return to baseline further impacting our results.

Patients with RLS often exhibit a tendency toward hypertension suggesting involvement of the Autonomic Nervous System. Izzi et al reported a reduced amplitude of both sympathetic and parasympathetic responses to the Head Up Tilt Table Test (HUTT) in RLS patients [28]. PLMs might be evoked by activation in the brain accompanied by a sympathetic response. As the sequence is repeated many times each night, it may be considered a potential risk factor for nocturnal arrhythmias and hypertension, increasing the cardiovascular problems in these patients. Bertish has reported that PLMs are associated with a lower baroreflex gain and higher leg vascular resistance [29]. However, they did not find a relationship between the baroreflex gain and RLS intensity. The increases in vascular resistance may reflect the mechanism through which RLS contributes to increased cardiovascular risk. In the current study, we found a reduction of PLM power with GEn accompanied by a reduction in blood pressure changes in PLMs with and without arousals. It has been reported that PLMs may still contribute to blood pressure increases in the absence of arousals [30,31]. Autonomic activation/ suppression typically results in concurrent changes in heart rate and blood pressure. In this study, one patient paradoxically showed an increase in the number of PLMs and 5 had little change in PLMs frequency but showed a decrease in PLMs power with GEn administration despite reports of subjective improvement. Of the 11 patients who reported feeling moderately to a great deal better, 9 had a decrease in PLMs power and 2 no change in PLMs power.

Our study has several limitations relating to design, subject selection, and data analysis. First, we opted to use a single group design due to the small sample size of the study. Secondly, we did not obtain a "real baseline", but instead started the study with a placebo baseline due to budgetary constraints. While the use of a placebo probably has little effects on PLMs, placebo may impact sleep and subjective parameters. Further, since patients with moderate to severe RLS with PLMI of 10 episodes per hour present with complaints of poor disrupted sleep, we selected 10 as a cutoff for inclusion. Whereas we demonstrated GEn's dampening effects on systolic blood pressure, we did not assess diastolic pressure effects. In addition, we did not assess the individual impact of PLMs with arousals on BP as opposed to PLMs without arousals. Also, the use of the second of two consecutive nights in the sleep lab for establishing baseline and one- month assessments may have impacted the degree of change observed with GEn. While we intended to eliminate any contributions from the well-established first night effect of adaptation and re-adaptation to the sleep laboratory, it generally takes 3 nights to establish normative sleep data [32,33]. Ideally, a third consecutive night for establishing baseline and drug effects would more accurately reflect GEn's effect on sleep architecture. It is not clear whether a first night effect impacting sleep stages and sleep quality would influence RLS frequency and intensity. Lavoie showed that heart rate and EEG responses to PLMs differ between sleep stages as well as during wake [34]. These differences may be exacerbated with a first night effect. Lavoie also reported an increase in the delta and theta band power occurring prior to the onset of each PLM in light stages 1 and 2 sleep whereas, in REM sleep there was an increase in higher EEG frequencies after the PLM event. Thus, sleep seems to deepen (greater delta power) prior to the PLMs followed by an inevitable lightening of sleep ranging from fast EEG activity to arousal. Sforza reported that 60% of PLMs were associated with microarousals with clear predominance in light stages 1 and 2 while PLMs without arousal predominated in slow wave sleep [35]. These data suggest that cardiovascular effects of PLMs are impacted by sleep stages and depth of sleep. Finally, whereas we excluded conmeds that are psychoactive, we did not require subjects to withdraw from any hypertensive meds despite their possible impact on heart rate and BP. Only tow subjects who completed the study were taking antihypertensives.

Conclusion

In conclusion, our exploratory study suggests that the transient increases in blood pressure and EMG power associated with nocturnal PLMs and EEG activation with arousal can be mitigated or ameliorated with GEn through its depressant effects on the central nervous system. As we reported previously in a smaller cohort, this suggests that the power of PLMs in RLS patients may me a more useful measure than simply PLM frequency or associated arousals in predicting blood pressure changes that, over time, may impact cardiovascular health and including the power of PLM in PSG analysis may provide a better measure of clinical consequences of this disorder. The significance of such fluctuations is difficult to establish with the available data given the small sample size. Future projects should expand the study population to better determine whether long term GEn use can decrease nocturnal hypertension and cardiovascular risk in patients with RLS.

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