Clinical Study Report

REBOX

Study EudraCT number: 2019-004917-15

22/10/2021

CONFIDENTIAL

1 TITLE PAGE: Crossover, Double-blind, Phase 2 Study of a Fixed Dose Combination of Reboxetine\Oxybutynin (AD128) Versus Placebo in Obstructive Sleep Apnea (RebOx)

Name of Test Drug: Reboxetine and Oxybutinyn

Indication studied: Obstructive sleep apnea

Study description:

Sponsors: Istituto Auxologico Italiano IRCCS

Protocol: Protocol V2, March 30th 2020

Clinical Phase: II

Study dates: end of the study 31\10\2021

PI: Dr.ssa Elisa Perger

GCP Statement: This study was performed in compliance with ICH Good Clinical Practise (GCP) including the archiving of essential documents

Date of report: 29\10\2021

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3 METHODS

3.1 Patients

Both male and female patients between 18 and 70 years of age with a recent (<1 year) diagnosis of OSA were eligible for study enrollment. Subjects treated with CPAP were included in the study (Table 1) only if they showed poor compliance (use of CPAP less than 4 hours per night for 70% of nights) and they were asked to completely stop the treatment at least 2 weeks prior to the baseline PSG. Exclusion criteria included any clinically significant neurological, psychiatric or cardiovascular disorder, untreated narrow angle glaucoma, hypertension requiring more than 3 drugs to be controlled, use of respiratory stimulants or depressants, hypnotics, central nervous system stimulants or other medicaments known to interact with study drugs, central sleep apnea, pregnancy, history of benign prostatic hyperplasia or urinary retention, which may be exacerbated by antimuscarinic medications.

Participants were enrolled from July 2020 to October 2020 through our sleep clinic (Istituto Auxologico Italiano, Milan, Italy) after a pre-screening evaluation for inclusion and exclusion criteria based on the clinical history. The trial ended when the previously calculated sample size was reached.

The study was approved by the Ethics Committee and by the Italian drug agency AIFA (Agenzia Italiana del Farmaco). Informed consent in writing was obtained from all study participants. The study was registered at ClinicalTrials.gov (NCT04449133).

3.2 Study Design

This was a randomized, double blind, placebo-controlled, cross-over, phase II, single center efficacy study of the combination of reboxetine and oxybutynin in adults with OSA.

Study participants underwent further eligibility screening with a one-night in-lab baseline in-lab PSG (Embla, Reykjavik, Iceland), which served as the baseline for AHI and other PSG endpoints. Participants were eligible for randomization if AHI on baseline PSG was>15 events/hr. Eligible participants were then randomized equally to first receive 4 mg reboxetine plus 5 mg oxybutynin (reb–

oxy) or matching placebo (2 capsules). Subjects started taking study drug at home the day after the baseline PSG immediately prior to bedtime for 7 days. A washout of 7-10 days preceded the switch to the other arm of the study. During the entire at-home period (6 nights on placebo and 6 nights on reb-oxy), the patients underwent full night pulse-oximetry testing (Nonin Medical Inc., 3150, Minnesota, USA). On the final night of dosing for each arm, participants performed an in-lab PSG to evaluate OSA severity. The predefined primary outcome variable was the change in AHI from baseline. Secondary outcomes were: response rate based on ≥50% reduction in AHI; proportion of participants with AHI<15/hour; change in subjective sleepiness with Epworth Sleepiness Scale (ESS), Psychomotor Vigilance Test (PVT), change from baseline in these PSG parameters: Oxygen Desaturation Index (ODI) at 3% threshold and hypoxic burden. Karolinska Sleepiness Scale (KSS), Patient Global Impression of OSA Severity (PGI-S), arousal index, periodic limb movement (PLM) index) and descriptive summary of nightly change with at-home pulse oximetry (ODI 4%) were also assessed.

3.3 Randomization and blinding

Study medications were prepared by the ST Pharma PRO SRL (Milan, Italy) and were placed in identical capsules that could not be identified by study personnel or participants. Participants were randomly assigned in a 1:1 equal allocation ratio to receive the active treatment dose or placebo first using a blocked randomization (block size of 2). Each participant was assigned a unique number (randomization number) that encoded the participant's assignment to 1 of the 2 arms of the study. The randomization list was produced and validated by a statistician not involved in patient recruitment and external to the hospital. No stratification was expected for any characteristics. Subjects, care providers, investigators, and outcomes assessors were blinded to the treatment allocation (quadruple blinding). Study treatment was dispensed the morning after PSG screening. Once all data analyses were completed and reviewed, the database was locked and the intervention allocations were unblinded for statistical analysis

3.4 Data analyses and measurements of outcomes

Overnight PSG recordings and scoring were performed in accordance with the American Academy of Sleep Medicine (AASM) rules[1]. All studies were scored by the same specialized sleep clinician, blinded to treatment assignment, according to AASM criteria[2]. AHI, ODI 3%, arousal index, and PLM index were calculated from the PSG. The OSA specific hypoxic burden (respiratory event–related oxygen desaturation area under pre-event SpO_2 baseline curve, per hour) was also calculated[3,4]. ODI at 4% threshold level (ODI 4%) was collected during at-home pulse oximetry for each night of treatment. Adverse events were recorded at each visit.

Pathophysiological traits causing sleep apnea were (endotypes) estimated during NREM sleep using established automated methods and executed using custom software (Endo-Phenotyping Using Polysomnography; MATLAB, Mathworks, Natick MA)[5-7]. For details please refer to supplement material.

The ESS questionnaire was taken to evaluate subjective somnolence over the preceding week of treatment[8] and the KSS was taken to measure the situational sleepiness in the late afternoon before the in-lab PSG. The PGI-S was used to rate the participants impression of disease severity. A validated 3-minutes PVT evaluated the sustained-attention and reaction-time by measuring the speed with which subjects responded to a visual stimulus[9,10]. The reaction time (RT), the number of lapses (defined as RT > 500 ms, i.e. inability to respond in a timely fashion when a stimulus was present and the reciprocal RT as a measure of speed (1/RT) (lapses included) were studied. The above-mentioned evaluations together with respiratory rate, EKG and three measurements of blood pressure, were performed without coffee intake in the previous 3 hours and at the same time of the day before the PSG.

3.5 Pathophysiological traits causing sleep apnea

Briefly, each trait is defined by spontaneous fluctuations in ventilation (from nasal pressure, mean-normalized) and ventilatory drive (intended ventilation estimated using a chemoreflex model and least-squares regression). Collapsibility was based on the median ventilation during sleep at normal/eupneic ventilatory drive (Vpassive); lower values of Vpassive indicate greater collapsibility[5]. Compensation, the increase in ventilation with rising ventilatory drive, was determined by calculating Vactive (ventilation when ventilatory drive is at the arousal threshold); greater Vactive, for any given Vpassive, reflects greater dilator muscle compensation. Loop gain (LG1, ventilatory control sensitivity) was determined from the ventilatory drive response to reduced ventilation; higher values represent a greater ventilatory control instability. Arousal threshold was measured as the ventilatory drive preceding each scored arousal[6]; low values reflect greater arousability.

3.6 Statistical analysis

Individuals were enrolled until 16 completed baseline and both treatment nights. The study was powered to detect an AHI reduction with reboxetine plus oxybutynin (percent reduction from baseline) by 50+/-50 % more than placebo (alpha 5%, power 80%); SD of the effect was estimated from a previous trial[11].

Data are presented as median [interquartile range]. Continuous variables were compared using a two-tailed Wilcoxon matched-pairs signed-rank test. Categorical data were analyzed using Fisher's exact test.

For the endotypic traits, the effect of the reb-oxy combination and placebo vs baseline were modelled by using linear mixed effects models, with treatments as fixed effects and subjects as a random effect. See supplemental material for further details. Effects on Vpassive (collapsibility) were modelled by using a sigmoidal transformation function (slope of 1 at Vpassive = 50%) to handle the known floor and ceiling effects[5] ; changes in collapsibility using our method are only linearly related to underlying collapsibility in the flow-limited range between Vpassive = 0% (apnea) and Vpassive =

100% (open airway). Effects on muscle compensation were estimated by modelling Vactive (same sigmoidal function) while adjusting for Vpassive. Effects on LG and arousal threshold were modelled by using simple linear models.

The effects of placebo and reb-oxy on repeated measures of ODI 4% at home were analyzed using a mixed effects model testing treatment, time, and the interaction between treatment and time as fixed effects and subjects as random effects. Comparisons between ODI on reb-oxy *vs.* placebo at individual time points (days 1-6) were corrected for multiplicity using the Sidak method.

To evaluate the predictors of response to reb-oxy from baseline characteristics, we performed a univariate linear regression analysis including baseline age, BMI, PSG characteristics (AHI, ODI, fraction of events that were hypopneas, mean desaturation associated with an event) and each endotype as independent variables. The percent change in AHI was the dependent variable. Associations were exploratory and were not adjusted for multiple comparisons.

Regarding baroreflex sensitivity, ambulatory blood pressure and heart rate variability Continuous variables were compared as change from baseline after 1-week of placebo and 1-week of reb-oxy using a two-tailed Wilcoxon matched-pairs signed-rank test.

A p-value of <0.05 was considered statistically significant. Statistical analyses were performed using Graph Pad Prism 6.0 (McKiev Software, Boston, MA) and MATLAB (MathWork).

4 RESULTS

4.1 Subjects

Eighteen subjects were enrolled in the study and performed a baseline PSG night; all individuals were eligible for randomization based on AHI>15 events/hr (Consort diagram in Figure 1). One subject dropped out prior to starting the first treatment period (second wave of COVID-19 in Milan; active drug period). One subject dropped out at the end of the first treatment period (also active drug period) as the patient was unable to continue (personal problems).

Data from 16 participants were available for analysis of OSA severity at baseline and on both nights after the week of drug or placebo intake. The characteristics of these subjects are shown in Table 1. None had previous history of upper airway surgery.

The results relative to primary and secondary outcomes were upheld when adjusted for sequence and period effects in a linear mixed effect model analysis (see paragraph 4.10). A significant sequence effect was found in the analysis of AHI %reduction. Adjusted results showed a reduction of placebo effect, suggesting a possible mild carry-over effect on placebo when it was administered after reboxetine plus oxybutynin, see the supplement for the detailed model. Secondary outcomes such as HB or PVT were not affected by period or sequence.

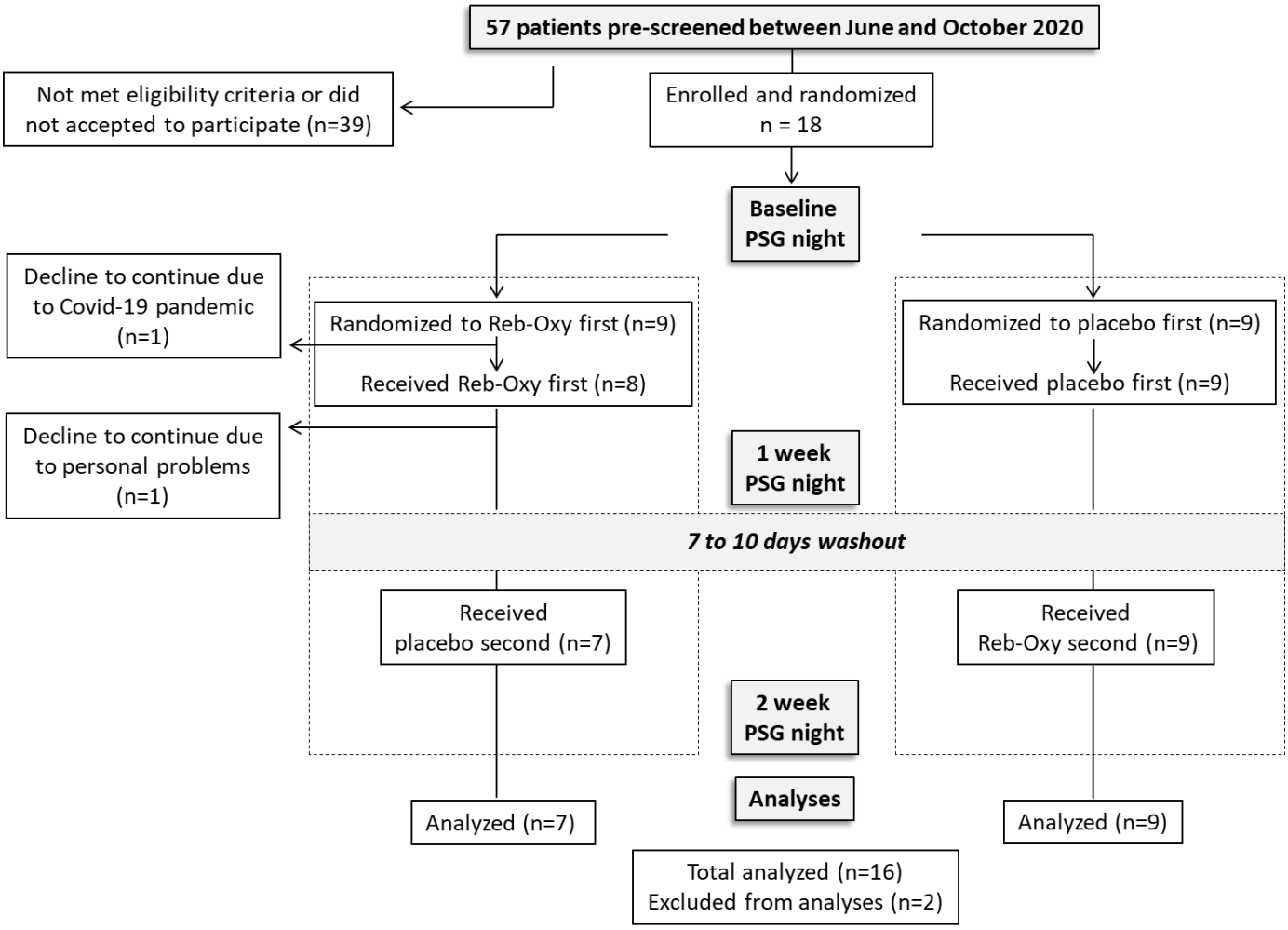

***Figure 1**: Consolidated Standards of Reporting Trials diagram of the clinical trial.*

***Table 1**: General characteristics of the population studied*

CHARACTERISTICS	VALUES
Age, years	57 [51-61]

Male, N (%)	14 (87.5)
Height, cm	180 [171-184]
Weight, Kg	94 [77-105]
BMI, Kg/m^2	30 [26-36]
Waist circumference, cm	116 [103-123]
Neck circumference, cm	43 [39-46]
Mallampati score (1 / 2 / 3 / 4)	1 (6.3) / 10 (62.5) / 4 (25) / 1 (6.3)
Tonsils score (1/ 2 / 3 / 4)	15 (93.7) / 1 (6.3) / 0 / 0
Smoke	8 (50)
Previous OSA treatment, n (%)	
C-PAP	5 (31.2)
Comorbidities, N (%)	
Hypertension	7 (44)
Diabetes	1 (6.3)
Dyslipidemia	7 (44)
Hypothyroidism	3 (18.8)
Rheumatoid arthritis	1 (5.6)
Medications, N (%)	
ACE-I/ARB	6 (35)
CCB	1 (6.3)
Diuretics	1 (6.3)
Antilipidemics	4 (25)
Antidiabetics	1 (6.3)
Antithrombotics	2 (12.6)

Definition of abbreviations: BMI = body mass index; OSA = obstructive sleep apnea; CPAP = continuous positive airway pressure; ACE-I = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; CCB = calcium channel blocker Data are expressed as number (%), median [interquartile range] unless otherwise specified

4.2 Effect of reb-oxy on AHI, oxygen saturation and sleep architecture

Reb–oxy reduced the AHI by a median of 26 events/h, or 59% (expressed as the median value of all reductions), compared with baseline and by 20 events/h, or 59% compared to placebo (Table 2; see Figure 2 for individual data). The vast majority of patients (81%) experienced a reduction in AHI > 50% on the treatment night, and 37% of the patients on reb-oxy had an AHI<15. Effects of the intervention on AHI specific to REM and NREM sleep stages, hypoxic burden, ODI, arousal index and sleep architecture are shown in Table 2. Reb-oxy significantly reduced hypoxic burden and ODI ($p<0.001$ and $p=0.021$, respectively). Considering that an hypoxic burden >53%min/h has been previously associated with higher cardiovascular-related mortality[19], reb-oxy reduced the hypoxic burden below this threshold in the 69% of our sample. Individual data on hypoxic burden are reported in Figure 3A and 3B. Reb-oxy significantly reduced the number of arousals compared to baseline and placebo, and sleep architecture was unchanged with the exception of a trend for reduced REM sleep and increased N2 on reb-oxy compared to placebo. No difference in periodic leg movements were observed among the three nights.

Table 2*: Obstructive Sleep Apnea Severity and Sleep Architecture Baseline, on Placebo and on Drug Combination for All the Participants (n = 16)*

	Baseline	**Placebo**	**Reb-Oxy**	**p-value**
AHI total, events/h	48.7 [34.8 to 56.6]	38.7 [29.0 to 47.8]	18.0 [12.5 to 21.4]	<0.001
%change from baseline		5.9 [-4.5 to 37.5]	59.2 [53.3 to 68.1]	<0.001
AHI supine, events/h	60.4 [52.7 to 81.9]	56.3 [44.9 to 76.0]	33.7 [25.3 to 48.1]	<0.001
%change from baseline		7.0 [0.4 to 27.2]	51.1 [30.9 to 64.3]	<0.001
Proportions of patients with AHI reduction>50% from baseline		13%	81%	<0.001
Proportion of patients with AHI<15 events/h		6%	37%	0.080
Hypoxic burden, %min/h	90.8 [69.5 to 154]	75.5 [68.1 to 168.0]	39.7 [25.4 to 55.3]	<0.001
%change from baseline		7.7 [-17.3 to 44.5]	61.5 [38.2 to 72.5]	<0.001

ODI 3%, events/h	42.7 [32.3 to 53.0]	36.8 [23.8 to 43.2]	31.4 [19.1 to 37.7]	0.021
%change from baseline		11.1 [-4.6 to 25.3]	29.0 [13.3 to 42.6]	0.025
ODI 4%, events/h	34.8 [23.9 to 43.9]	30.1 [17.4 to 40.0]	20.1 [13.3 to 28.2]	0.001
%change from baseline		7.7 [-7.7 to 38.2]	38.5 [21.1 to 49.7]	0.016
Arousal index, events/h	30.6 [20.7 to 47.7]	26.6 [14.1 to 34.7]	10.7 [7.6 to 16.8]	0.003
Total Sleep time, min	329.5 [301.0 to 368.8]	323.5 [274.4 to 351.4]	321.8 [283.0 to 362.9]	0.376
Sleep efficiency, %TIB	71.2 [59.9 to 76.2]	71.7 [60.8 to 83.5]	69.7 [64.0 to 73.3]	0.504
N1, %TST	3.7 [2.4 to 7.3]	3.5 [2.8 to 4.5]	5.4 [2.7 to 9.9]	0.102
N2, %TST	63.5 [55.3 to 68.1]	62.9 [58.5 to 68.7]	68.0 [58.4 to 75.8]	0.051
N3, %TST	16.2 [10.9 to 22.1]	17.4 [9.5 to 26.3]	15.9 [6.8 to 23.0]	0.117
REM, %TST	18.1 [13.8 to 21.4]	16.2 [13.2 to 17.9]	10.2 [5.1 to 15.5]	0.057
PLM index, events/h	0.0 [0.0 to 2.8]	0.0 [0.0 to 2.8]	0.5 [0.0 to 2.8]	0.457
Heart Rate, bpm	78 [71 to 90]	82 [72 to 93]	79 [69 to 87]	0.700
Systolic blood pressure, mmHg	133 [124 to 145]	126 [118 to 135]	120 [115 to 138]	0.234
Diastolic blood pressure, mmHg	82 [75 to 89]	84 [75 to 92]	80 [73 to 88]	0.065

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; AHI = apnea–hypopnea index; ODI = oxygen desaturation index; TIB = time in bed; N1-2-3 = non-REM stage 1-2-3; TST = total sleep time; REM = rapid eye movements sleep; PLM = periodic legs movements. Data are presented as median (interquartile range). % changes are expressed as the median of the group percentage change. P values compare placebo versus reb-oxy.

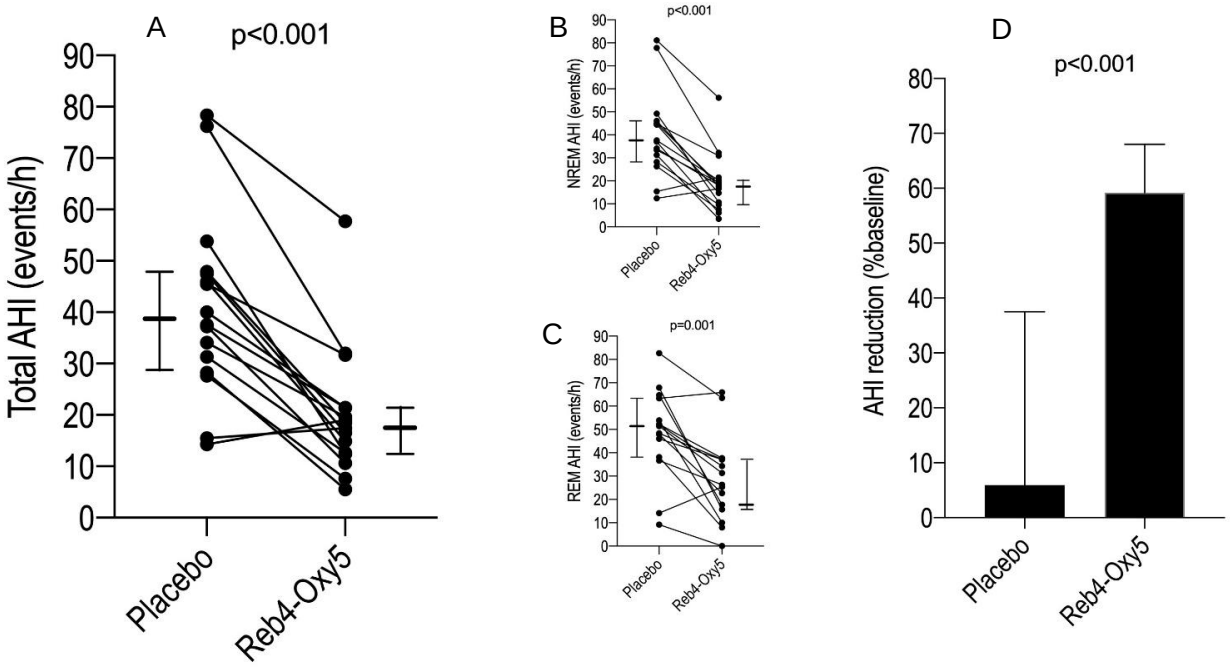

Figure 2: *Individual data showing the effect of reboxetine plus oxybutynin (reb–oxy) on (A) total apnea–hypopnea index (AHI), during NREM (B) or REM (C) sleep stages. Longer horizontal lines indicate median values, and shorter lines indicate 25th and 75th percentiles. (D) Group data showing percentage of apnea–hypopnea index (AHI) changes from baseline on placebo and on reb-oxy.*

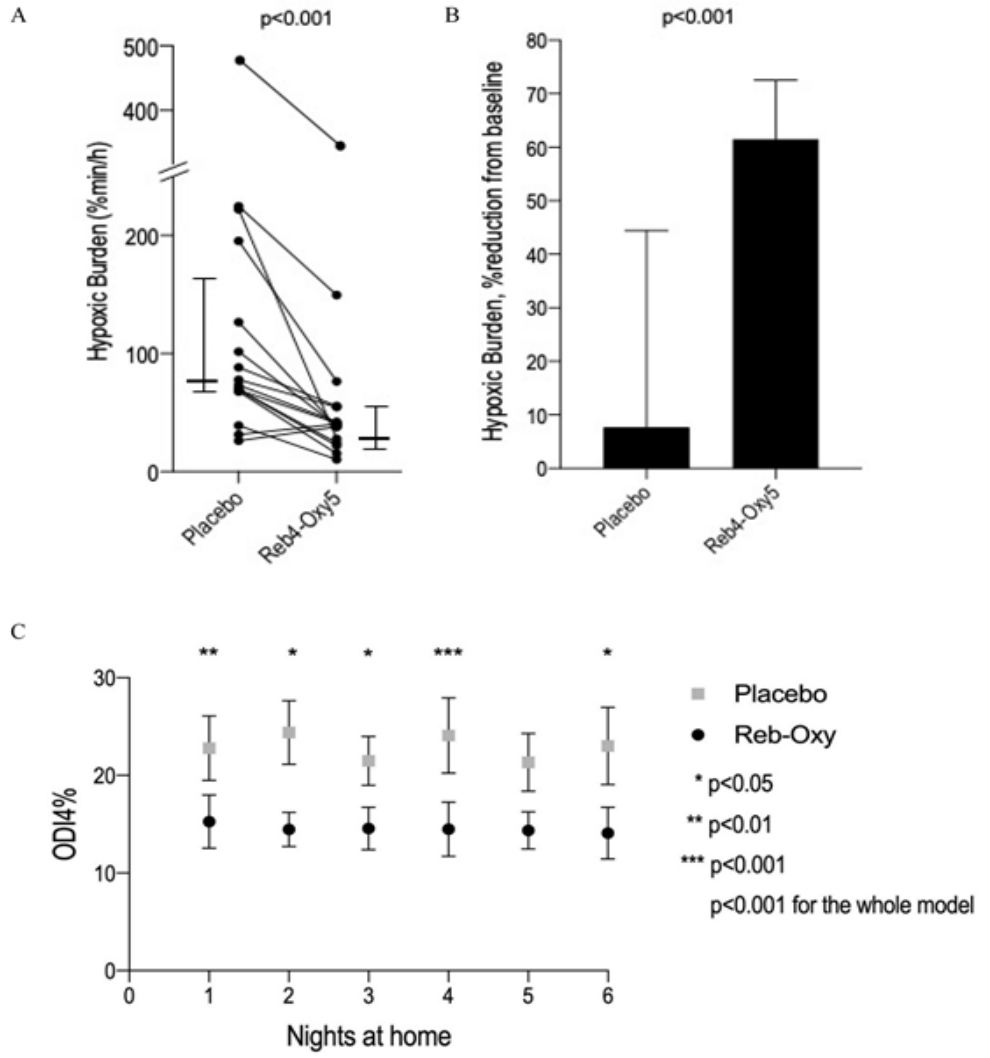

Figure 3*: Effect of reboxetine plus oxybutynin (reb–oxy) on desaturation index: (A) hypoxic burden as individual data. Longer horizontal lines indicate median values, and shorter lines indicate 25th and 75th percentiles. (B) Group data showing percentage of hypoxic burden changes from baseline on placebo and on reb-oxy are shown in panel. (C) Analysis of repeated measures of ODI 4% obtained during at-home pulse oximetry during placebo (grey squares) and during reboxetine plus oxybutynin (reb-oxy) weeks (black dots). Data were compared using a mixed effect model including treatment, time and time x treatment interaction as fixed effects and subjects as a*

random effect. Only treatment effect was significantly associated with ODI4% (dependent variable). P value for day-by-day multiple comparison between placebo and reb-oxy arms are adjusted using Sidak method.

4.3 Effect of reb-oxy on ODI at home

ODI 4% obtained during at-home pulse oximetry was collected on average (SD) 5.7 (0.8) nights on reb-oxy and 5.4 (1.0) nights on placebo. Group results are shown in Figure 3C for each night. In the mixed effects model, only treatment (reb-oxy vs placebo) was associated with a significant change in ODI 4% ($p<0.001$), while there were no effects related to time or to the interaction between time and treatment.

4.4 Effect of reb-oxy on subjective questionnaires and vigilance

Reb-oxy did not significantly improve subjective indices related to sleepiness, impression of disease severity or vigilance when considering group data (Table 3). Regarding subjective sleepiness, 4/5 patients with ESS>6 at baseline experienced improvement in the score from 11 [3 to 12.5] to 6 [1.5 to 6.5], although this did not reach statistical significance ($p=0.19$). PGI-S improved on reb-oxy compared to baseline, but again this difference did not reach statistical significance ($p=0.087$). Despite KSS revealing no change in subjective alertness between treatments, PVT as RT and 1\RT performance significantly improved on reb-oxy compared to placebo, as shown in Figure 4.

***Table 3**: Results of questionnaires regarding subjective indices related to sleepiness and impression of disease severity and objective vigilance test (n = 16).*

	Baseline	**Placebo**	**Reb-oxy**	**p-value**
ESS	5.0 [4.3 to 9.3]	5.0 [3.0 to 6.0]	5.0 [3.0 to 7.5]	0.75
%change from baseline		0 [-15 to 30]	25 [-10 to 42]	0.45
KSS	2.0 [1.0 to 2.8]	1.5 [1.0 to 3.0]	2.0 [1.0 to 2.8]	0.53
%change from baseline		0 [-75 to 25]	0 [-100 to 54]	0.75
PGI-S	7.0 [4.0 to 8.0]	4.0 [3.0 to 7.8]	3.5 [2.3 to 6.5]	0.184
%change from baseline		0 [-7 to 33]	21 [-14 to 56]	0.59
PVT, reaction time, msec	250 [239 to 312]	264 [217 to 284]	223 [172 to 244]	<0.001
%change from baseline		5 [-7 to 11]	19 [6 to 30]	0.02
PVT, lapses	2 (1.0%)	0	3 (1.6%)	0.33
PVT, 1/RT	4.0 [3.33 to 4.17]	3.8 [3.5 to 4.5]	4.5 [4.1 to 5.7]	0.02

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; ESS = Epworth Sleepiness Scale; KSS = Karolinska Sleepiness Scale; PGI-S = Patient Global Impression of OSA; RT: reaction time; Severity; PVT = Psychomotor Vigilance Test.

Data are presented as median [interquartile range]. % changes are expressed as the median of the group percentage change. P values compare placebo versus reb-oxy.

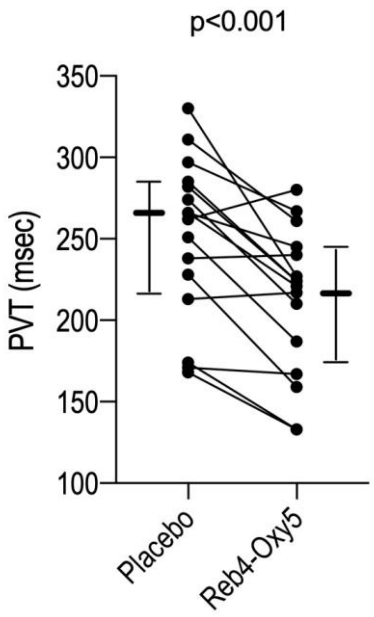

Figure 4: *Effect of reboxetine plus oxybutynin (reb–oxy) on Psychomotor Vigilance Test (PVT) reaction time. Longer horizontal lines indicate median values, and shorter lines indicate 25th and 75th percentiles.*

4.5 Effect of reb-oxy on pathophysiological traits

Group data from the mixed effects model of endotypic traits at baseline, on placebo, and on reb-oxy are shown in Table 4. Compared to placebo, reb-oxy increased muscle compensation by 30% of normal/eupneic ventilatory drive (eupnea), supporting the effect of this combination on UA muscle responsiveness. However, reb-oxy reduced the arousal threshold by 27% of eupnea, i.e. patients woke more easily on active treatment. Vactive was increased on reb-oxy by 20% of eupnea compared to baseline but not compared to placebo. No changes were found in loop gain (i.e. ventilatory control sensitivity) and Vpassive (i.e. passive pharyngeal tissue collapsibility).

***Table 4**: Mixed Effects Model for Effect of Reboxetine plus Oxybutynin vs Placebo on, Vpassive, Vactive, Muscle Compensation, Arousal Threshold, and Loop Gain During NREM Sleep*

Variable	Vpassive (%eupnea)	Vactive (%eupnea)	Muscle Compensation (%eupnea)	Arousal threshold (%eupnea)	Loop gain (unitless)
Intercept (Baseline)	82 [58 to 106]	76 [46 to 107]	-57 [-115 to 1]	139 [107 to 171]	0.60 [0.47 to 0.74]
Placebo vs baseline	+6 [-12 to 23] P=0.52	+20 [0 to 41] P=0.049	+11 [-6 to 27] P=0.198	+5 [-16 to 25] P=0.645	-0.01 [-0.13 to 0.11] P=0.879
Reb-oxy vs baseline	+17 [-4 to 38] P=0.11	+35 [10 to 60] P=0.007	+40 [17 to 63] P<0.001	-23 [-43 to -2] P=0.033	-0.09 [-0.21 to 0.03] P=0.15
Reb-oxy vs placebo	+11 [-9 to 32] P=0.259	+15 [-9 to 39] P=0.219	+30 [7 to 53] P=0.012	-27 [-48 to -7] P=0.01	-0.08 [-0.21 to 0.04] P=0.192

Data are presented as mean [95%CI]. Values for Vpassive do not represent observed data but rather the underlying collapsibility derived from a sigmoidal transformation function, to handle the ceiling effects previously described for these types of data[16]. Values for Muscle Compensation were calculated from Vactive adjusting for Vpassive such that the effect shown is the additional effect on ventilation above Vpassive (thus representing pharyngeal compensation).

4.6 Predictors from patients' baseline characteristics

We found an inverse relationship between the change in AHI and baseline mean desaturation, expressed as the average difference between the highest and lowest saturation value during respiratory events; the lower the desaturation, the higher the AHI reduction, r=-0.68, p=0.004. It was also found that the lower the arousal threshold, the higher the AHI reduction, r=-0.56, p=0.024. There was also a direct relationship between baseline Vpassive and AHI reduction: the higher the Vpassive (better airway anatomy), the greater the AHI reduction, r=0.5, p=0.047.

4.7 Linear mixed model effect on primary outcome

Table 5 and 6 shown the **l**inear mixed effect model for AHI. The significant sequence effect suggests that there was a trend for an increased %reduction in AHI from baseline on placebo when the active treatment was administered first (sequence 1, carry-over effect). In order to explore this possibility, we analyzed the patients separately, based on treatment sequence. Figure 5 shows a significant difference between placebo and active treatment in the %reduction of AHI after dividing the patients according to treatment sequence (top graphs). Bottom graphs show a significant increase in %reduction from baseline on the placebo arm on sequence 1 vs sequence 0, but no significant difference between sequences in the Reb-Oxy arm. No period or sequence effects were found in the analysis of the other outcomes.

Table 5. *Linear mixed effect model for apnea hypopnea index (AHI, percent reduction from baseline)*

Fixed effects:	**Mean [95% CI]**	**P-value**
Placebo	11% [-2 to 24]	0.09
Reb-Oxy (*Change from Placebo)*	+48% [31.5 to 64]	<0.001

Table 6. *Linear mixed effect model for apnea hypopnea index (AHI, percent reduction from baseline) accounting for sequence and period*

Fixed effects:	**Mean [95% CI]**	**P-value**
Placebo	1% [-12 to 14]	0.87
Reb-Oxy (*Change from Placebo)*	+46.5% [31 to 62]	<0.001
Period	+10% [-6.5 to 25.5]	0.2
Sequence	+25% [8 to 41]	0.005

Periods in the model were represented by the following values: -0.5 for Visit 1, 0.5 for Visit 2. Sequences in the model were represented by the following values 0: Placebo first, Reb-oxy second; 1: Reb-oxy first, placebo second.

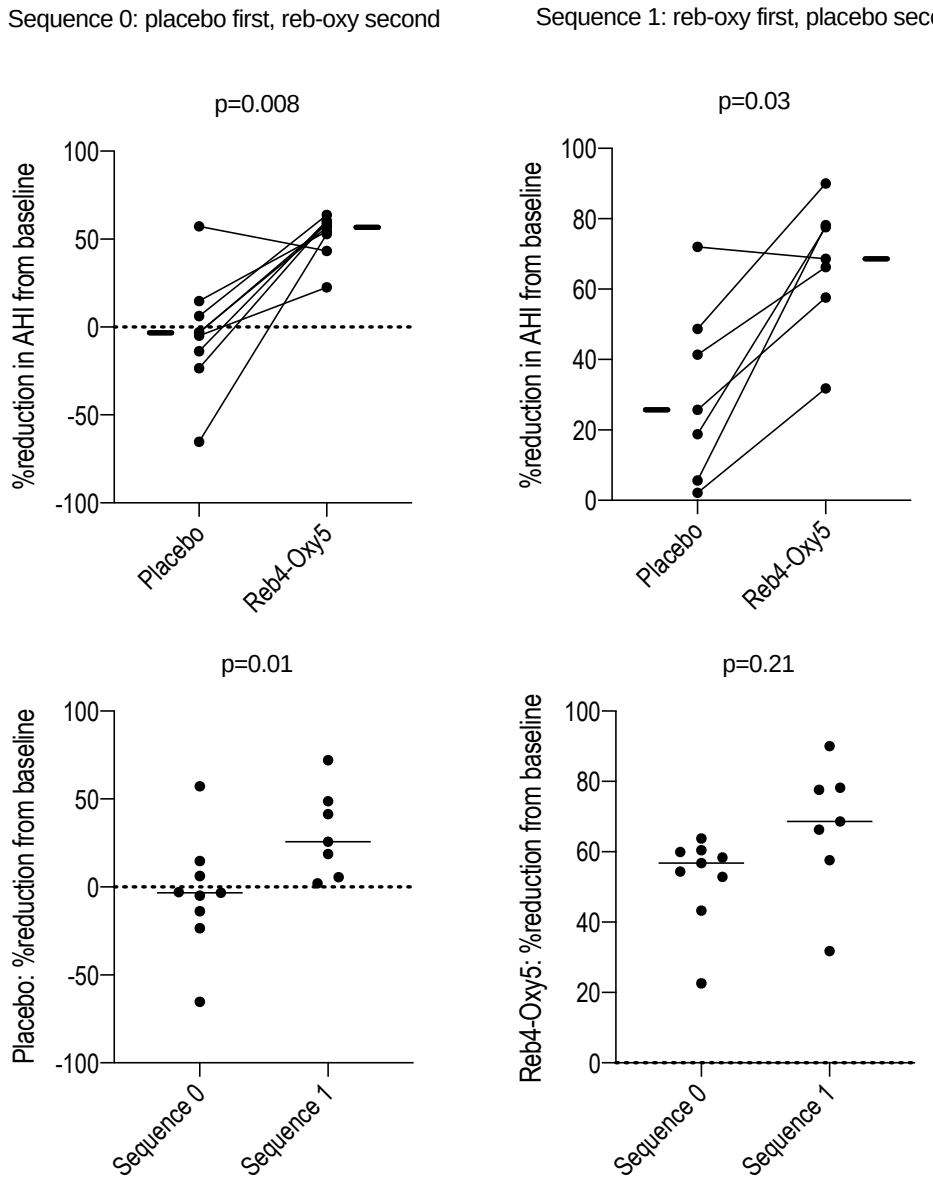

Figure 5. *Lines indicate medians; sequence 0: placebo first, then reb4-oxy5; sequence 1: reb4-oxy5 first, then placebo.*

4.8 Heart rate and blood pressure

Heart rate (HR) during the PSG increased from 65 [60-69] bpm at baseline to 69 [64-77] bpm on reb-oxy and to 66 [59-70] bpm on placebo (p=0.02) (Table 7). However, 24h HR measured during the ABPM was not different among treatment groups as shown in Table 7.

Reb-oxy did not significantly modify 24 h, daytime and night-time DBP and SBP (Table 8). Morning surge was not increased in reb-oxy versus placebo and blood pressure variability did not change during the day and the night between groups.

Neither in the time domain nor in the frequency one, reb-oxy administration was associated with any modification in HRV (Table 7).

Table 7*: Obstructive Sleep Apnea Severity, main sleep characteristics and heart rate variability at baseline, on placebo and on drug combination (n = 16). Heart rate variability data calculated from nocturnal PSG with 1 channel EKG during N2 sleep at baseline, on placebo and on drug combination. P-values are calculated as the percentage change from baseline in placebo versus reb-oxy.*

	Baseline	**Placebo**	**Reb-Oxy**	**p-value**
HR during full night PSG, bpm	65 [59.5 - 69]	65.6 [58.8 - 69.55]	69.35 [63.8 - 76.75]	0.02
RMSSD, ms^2	40.4 [17.5-51.6]	24.7 [17.6-43.1]	32.4 [25.4-51.6]	0.38
pNN50, ms^2	0.183 [0.01-0.32]	0.02 [0.001-0.18]	0.10 [0.03-0.35]	0.40
HF, ms^2	300.0 [90.7-748.4]	189.7 [85.6-527.2]	328.8 [181.5-887.9]	0.53
LF, ms^2	0.7 [0.4-0.8]	0.6 [0.5-0.7]	0.5 [0.4-0.6]	0.38
LF/HF, ms^2	2.4 [0.9-3.9]	1.3 [0.9-2.4]	1.1 [0.6-1.7]	0.46
VLF, ms^2	680.6 [341.1-2879.1]	504.4 [232.3-2095.3]	659.4 [434.4-885.6]	0.25

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; HR = heart rate; SD = standard deviation; RMSSD = Root Mean Square of the Successive Differences; pNN50 = the

proportion of number of pairs of successive NN (R-R) intervals that differ by more than 50 ms; HF = high frequency; LF = low frequency; VLF = very low frequency. Data are presented as median (1^{st}-3^{rd} quartiles). % changes are expressed as the median of the group percentage change. P values compare placebo versus reb-oxy.

***Table 8**: Ambulatory blood pressure data at baseline, on placebo and on drug combination (n = 16). P-values are calculated as the percentage change from baseline in placebo versus reb-oxy.*

	Baseline	**Placebo**	**Reb-Oxy**	**p-value**
DBP 24h, mmHg	83.0 [76.3-87.8]	83.0 [75.3-85.7]	79.6 [75.6-89.5]	0.68
SBP 24h, mmHg	131.6 [122.1-140.3]	129.5 [121.0-133.1]	121.5 [115.4-140.9]	0.72
Day-time DBP, mmHg	86.8 [82.2-92.6]	85.78 [80.4-88.3]	82.18 [78.6-94.7]	0.86
Day-time SBP, mmHg	138.7 [129.9-145.1]	133.0 [125.1-138.1]	125.0 [117.3-144.3]	0.46
Nocturnal DBP, mmHg	71.8 [65.9-76.0]	70.7 [67.1-76.5]	69 [65.6-76.5]	0.50
Nocturnal SBP, mmHg	112.3 [106.1-124.2]	118.5 [108.6-126.2]	110.1 [107.6-132.3]	0.28
ABPM 24h HR, bpm	78.0 [71.7-90.4]	80.2 [71.5-87.6]	79.1 [69-86.6]	0.60
DBP Morning surge	20.0 [15.5-40.0]	15 [6.5-34.5]	20 [10-33]	0.60
SBP Morning Surge	48.0 [31.5-87.5]	33 [23.5-45.5]	32.0 [25.5-39.5]	0.38
DBP variability day	18.1 [11.5-20.6]	15.7 [12.7-18.3]	15.7 [11.7-19.1]	0.90
SBP variability day	17.9 [13.9-24.9]	16.9 [12.2-21.1]	17.2 [15.7-19.8]	0.79
DBP variability night	9.9 [7.9-13.2]	10.6 [8.4-13.7]	9.6 [9.3-10.9]	0.23
SBP variability night	12.1 [10.4-13.8]	13.2 [11.2-17.3]	12.3 [9.1-16.6]	0.08

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin; DBP = diastolic blood pressure; SBP = systolic blood pressure.

4.9 Baroreflex sensitivity

Table 9 shown the baroreflex sensitivity and baroreflex resonance baseline and after 1 week of placebo and after 1 week of reb-oxy concerning. We found an increase of the baroreflex sensitivity during clinostatism after reb-oxy administration vs placebo, which expresses an effect of active treatment on the vagal component of cardiac activity mainly during clinostatism.

Regarding the baroreflex resonance (which is the pressure oscillation determined by the resonance of the baroceptorial reflex), we found a power spectral density reduction of diastolic blood pressure in the low frequency both in clinostatism and orthostatism on reb-oxy, which means a reduction of the sympathetic vascular tone due to active treatment.Reb-oxy did not induce orthostatic hypotension or other modifications of the parasympathetic activity indices.

***Table 9**: Baroreflex sensitivity and resonance at baseline, on placebo and on reb-oxy (n=16). P-values are calculated as the percentage change from baseline in placebo versus reb-oxy.*

	Baseline	**Placebo**	**Reb-Oxy**	**p-value**
***Baroreflex sensitivty*, ms/mmHg**				
Alpha_LF clinostatism	5.3 [3.8-8.6]	6 [4.2 -- 8.8]	9.3 [7.8 -- 10.8]	**0.02**
Alpha_LF orthostatism	2.6 [2.0-4.6]	3.1 [2 -- 4.3]	3.7 [2.5 -- 6.8]	**0.03**
H_LF clinostatism	3.9 [2.8-6.6]	5.1 [3.5 -- 6.8]	8.2 [5.8 -- 10.5]	**0.01**
H_LF orthostatism	2.0 [1.6-4.0]	2.2 [1.5 -- 3.3]	3.1 [1.9 -- 4.8]	0.07
BRS clinostatism	6.7 [4.2-7.6]	7.2 [3.3 -- 9]	9.3 [8 -- 12.6]	0.29
BRS orthostatism	3.9 [2.3-5.3]	3.4 [3.2 -- 4.5]	3.9 [2.4 -- 5]	0.08
***Baroreflex resonance*, mmHg^2**				
SBP LF clinostatism	7.8 [3.9-14.2]	9.3 [4.7 -- 11.7]	2.8 [2.1 -- 3.8]	**<0.01**
SBP LF orthostatism	14.6 [11.2-20.4]	17 [10.9 -- 21.4]	5.8 [4.2 -- 9.8]	**<0.01**
DBP LF clinostatism	2.7 [1.9-5.3]	3.5 [1.8 -- 5.8]	1.3 [1.1 -- 2]	**<0.01**
DBP LF orthostatism	5.0 [3.8-9.2]	5.9 [4.1 -- 9.4]	2.4 [1.7 -- 3.4]	**<0.01**

Data are expressed as percentage or median [interquartile]. The BRS was estimated with transfer function. Definition of abbreviation: BRS: baroreflex sensitivity; PSD = power spectral density; SBP = systolic blood pressure; DBP= diastolic blood pressure; LF = low frequency.

4.10 Side Effects

The following side effects were reported during the study on the reb–oxy night: urinary hesitation (difficulties in initiating micturition in the morning n=7 males); dry mouth during the night and in the morning (n=10); sexual dysfunction (erectile dysfunction in the morning or decreased libido n=3 males); brief sensation of palpitation (n=1) and insomnia symptoms (difficulty initiating and maintaining sleep; n=1). On placebo, chest pain (n=1) and side pain (n=1) were observed. No participants experienced severe side effects or severe adverse events in either arm. No differences were found in terms of resting blood pressure, heart rate, or EKG among the visits. Side effects on reb-oxy and placebo are presented in Table 9.

Table 9*: Adverse Events (AE) during the week on placebo and the week on reboxetine plus oxybutynin (reb-oxy). None reported severe AE. Comparisons were performed using a Chi-squared test (n = 16).*

	Placebo	**Reb-oxy**	**p-value**
Dry mouth	0	10	<0.01
Urinary hesitation	0	7	0.03
Sexual disfunction	0	3	0.69
Palpitation	0	1	0.31
Insomnia	0	1	0.31
Chest pain	1	0	0.31
Side pain	1	0	0.31
Headache	1	0	0.31

Cramps	1	0	0.31
Total n of patients reporting AE:	2	13	<0.01

Definition of abbreviations: reb–oxy = reboxetine plus oxybutynin;

Data are presented as number (percentage).

5 OVERALL CONCLUSIONS

This study provides experimental evidence that reboxetine plus oxybutynin administered before bedtime substantially reduces OSA severity (AHI) after 1-week of treatment. In addition to the AHI reduction, reb-oxy also exerted a significant effect on indices of hypoxemia, such as ODI and hypoxic burden. Reb-oxy also improved the performance on the vigilance testing. OSA alleviation was likely mediated by improved UA muscle activity and responsiveness, as suggested by the ~30% increase in muscle compensation on the drugs. Home pulse-oximetry recordings showed that reb-oxy was effective at improving nocturnal oxygen saturation as early as the first day of treatment, likely due to reduced OSA severity, and its efficacy was maintained through the 7th day, as shown in the in-lab PSG. The combination of reboxetine plus oxybutynin did not increase blood pressure both during the day and during the night and did not increase the cardiac sympathetic modulation as reflected by HRV during the night. The baroreflex sensitivity increased and SBP and DBP oscillations at LF (expression of resonance in the baroreflex loop) decreased, pointing out a decreased sympathetic modulation of the vascular resistances. Orthostatic hypotension was not observed. To date, this is the first evaluation of the impact of the new OSA pharmacologic therapy on cardiac autonomic system.

The current study showed for the first time that repeated doses of the combination of noradrenergic and anti-muscarinic drugs is efficacious for the alleviation of OSA. Specifically, over one week, reboxetine plus oxybutynin provided a 59% reduction in AHI, and halved OSA severity in 81% of individuals. Acute effects exhibited on the first night were sustained at the end of the week. The administration of a noradrenergic drug (reboxetine) plus oxybutynin did not induced clinically relevant sympathetic overactivity and, together with a reduction in OSA severity, increased baroreflex sensitivity. While subjective sleepiness was not reduced in this population, objective psychomotor vigilance test showed promising signs of improvement without major safety issues. These results provide strong pilot data for the design of larger and longer studies testing these drugs as a pharmacological therapy for OSA patients.

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