Online only supplementary material

Effect of mandibular advancement therapy on inflammatory and metabolic biomarkers in patients with severe obstructive sleep apnoea: A randomized controlled trial.

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Methods (expanded version)

Study Design and Patients

This randomized, single-blind, parallel-group trial conducted in five French sleep centres was approved by our local ethics committee (Comité de Protection des Personnes, Ouest II, Angers; No. 2010/14), and registered with ClinicalTrial.gov (NCT01426607). Patients with severe obstructive sleep apnoea (OSA; Apnoea-Hypopnoea Index [AHI] > 30), aged 18-70 years, for whom mandibular advancement device (MAD) therapy was considered as second-line therapy because of continuous positive airway pressure (CPAP) intolerance, were assessed for eligibility. Exclusion criteria were body mass index (BMI) greater than or equal to 32 kg/m^2; history of cardiovascular disease (CVD) including coronary heart disease, heart failure, arrhythmias, and stroke; coexisting sleep disorders other than OSA; central sleep apnoea defined by a central apnoea index greater than or equal to 5; severe daytime sleepiness defined by an Epworth Sleepiness Scale greater than or equal to 16; and inadequate dental structure or temporomandibular joint disease contraindicating MAD treatment as assessed by a dentist. All patients provided their written informed consent to participate in the study.

Randomization

Patients were randomly assigned to receive 2 months of treatment with either effective MAD or a sham device according to a 1:1 allocation using a computer-generated randomization list stratified by site with permuted blocks of random sizes. The effective MAD was custom-made, consisting of an adjustable two-piece acrylic oral appliance (AMO; Orthosom, Beaucouzé, France) with attachments of various sizes allowing adjustment of mandibular advancement (Figure E1). The sham device consisted of the upper appliance only and did not advance the mandible. As previously described,[1] treatment adherence with the effective MAD and the sham device was objectively measured by a validated embedded microsensor thermometer (TheraMon®, IFT Handels- und Entwicklungsgesellschaft GmbH,

Handelsagentur Gschladt, Hargelsberg, Austria). With ethics committee approval, patient blinding was achieved by concealing the less effective nature of the sham device.[1,2]

Interventions

At baseline, patients underwent clinical assessment, overnight in-lab polysomnography (PSG) followed by collection of blood samples. All patients underwent a 6-week MAD acclimatization period, during which the mandible was incrementally advanced by 1-mm steps every 1 or 2 weeks until symptom relief or until adverse effects prevented further advancement. Patients were then submitted to a one-week washout period, after which they were allocated to receive 2 months of treatment with either effective MAD or the sham device. Clinical assessment, PSG with effective MAD or sham device, and blood sample collection were repeated after the 2-month treatment period. Objective treatment adherence was also calculated after the 2-month treatment period.

Measurements of inflammatory and metabolic biomarkers

After overnight fasting, blood samples were collected in EDTA tubes (Vacutainers, Becton Dickinson, Le Pont de Claix, France) from a peripheral vein using a 21-gauge needle to minimize platelet activation, and were processed for assays within 2 hours. Samples were centrifuged for 20 minutes at 250 g. Platelet-rich plasma was harvested and centrifuged 20 minutes at 1500 g to obtain platelet-free plasma (PFP). PFP was aliquoted and stored at 80°C for subsequent use.

The assessment of inflammatory biomarkers was performed on PFP with an enzyme-linked immunosorbent assay (ELISA) using MESO QuickPlex SQ 120 assay (MSD, Rockville, Md., USA). Each assay was performed in duplicate in order to verify the intra-assay variability using samples randomly prepared. Then, if the coefficient of variability was less than 10%, the mean of each duplicate was calculated. Measures with a coefficient of variability > 10% were considered as unavailable biological data (n=5). A single assay was used to measure

plasma levels of adiponectin, C-reactive protein (CRP) and leptin. Multiplex assays were used for the assessment of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), N- terminal pro-brain natriuretic peptide (NT-ProBNP), tumor necrosis factor receptor 1 and 2 (TNF-R1 and TNF-R2) and P-selectin. Both assays depended on the electro chemiluminescent compound SULFO-TAG™ linked to a detection antibody. All the solutions were supplied by MSD and experiments were performed according to manufacturer's protocol.

For single assays, PFP was first 1000-fold diluted for adiponectin and CRP in an assay diluent whereas detection of leptin did not require any dilution. Then, the samples were incubated in 96-wells plate coated by a capture antibody directed against the protein of interest. After 2 hours of incubation at room temperature with vigorous shaking, the plate was washed three times with PBS supplemented with 0.05% of Tween-20. Then, SULFO-TAG-labelled detection antibodies were added for another 2 hours at room temperature with vigorous shaking. At the end, three other washes were performed before addition of a read solution. Then, voltage stimulation of plate electrodes induced chemilumescence read by the instrument (MESO QuickPlex SQ 120, USA). The level of the protein of interest is proportional to the emitted light present in the PFP and is calculated thanks to a calibration curve. The limits of detection (LOD) of CRP, adiponectin and leptin were 1.33 pg/mL, 0.005 ng/mL and 43 pg/mL respectively. None of the measured values were outside of these limits.

For the multiplex assay, samples were 2-fold diluted and then incubated for 2 hours with vigorous shaking in a 96-wells plate. Each well is seeded in 6 distinct spot coated with capture antibody specific of the protein of interest. After three washes with PBS supplemented with 0.05% of Tween-20, SULFO-TAG-labelled detection antibodies were added for another 2 hours at room temperature with vigorous shaking. At the end, three other washes were performed before addition of a read solution. Then, voltage stimulation of plate electrodes induced chemilumescence read by the instrument (MESO QuickPlex SQ 120, USA). The

level of the protein of interest is proportional to the emitted light present in the PFP and is calculated thanks to a calibration curve. The calculated LOD of IL-6, TNF-α, TNF-RI, TNF-RII, P-selectin and NT-proBNP were 0.06 pg/mL, 0.04 pg/mL, 0.569 pg/mL, 0.102 pg/mL, 29.9 pg/mL and 0.311 pg/mL respectively. None of the measured values were outside of these limits.

Plasma glucose, insulin, triglycerides, total serum cholesterol, and high-density lipoprotein serum cholesterol (HDL-c) were directly measured in accredited laboratories using standard techniques. Low-density lipoprotein serum cholesterol (LDL-c) was calculated. The homeostasis model assessment resistance index (HOMA-IR.) was calculated from fasting glucose and insulin concentrations, as follows: insulin (mIU/l) * glucose (mmol/l)/22.5.

Statistics

A sample size calculation was performed for the primary endpoints of the main study.[1] In the present ancillary study, we performed a per-protocol analysis including patients with available biomarkers before after 2 months of effective MAD or sham device. Continuous variables were described as mean (SD) or mean (95% confidence interval [CI]) for variables with a normal distribution and as median (interquartile range) for variables with a non-normal distribution. Normality of distribution was assessed using the Kolmogorov–Smirnov test. Normal variables were analysed using an unpaired Student's t test for intergroup difference and a paired t test for intragroup difference. Linear regression analysis was used to adjust for baseline values and potential covariates. Non-normal variables were analysed using the Mann-Whitney test for intergroup difference and the Wilcoxon signed rank test for intragroup difference. The Chi-square test and Fisher's exact test were used for categorical variables, as appropriate. All reported p values are two-sided. A p value less than or equal to 0.05 was considered to indicate statistical significance. All analyses were performed using STATA version 13.1 (STATA Corp., College Station, TX).

Results

Table E1: Baseline characteristics of all patients randomized in the main study[1] with patients included and not included in the present ancillary study.

	All randomized patients	Patients included in the ancillary study	Patients not included in ancillary study	p value
n	150	109	41	
Age, years	53.8 (10.2)	53.6 (10.1)	54.7 (10.7)	0.97
BMI, kg/m²	27.0 (3.2)	27.1 (3.3)	26.56 (2.9)	0.71
Women, %	14.4	12.1	23.1	0.48
Hypertension, %	20.7	20.9	17.8	0.51
Diabetes, %	5.1	5.7	3.6	0.71
Dyslipidemia, %	11.7	11.2	14.3	0.64
ESS	9.3 (4.2)	9.1 (4.2)	9.8 (4.3)	0.76
AHI, n	41.0 [35.0-53.0]	41.0 [34.0-52.0]	42.0 [36.0-53.0]	0.78
ODI, n	31.9 (17.9)	31.7 (17.1)	32.2 (19.9)	0.99
SBP, mmHg	125.7 (14.4)	126.4 (15.1)	123.6 (11.4)	0.66
DBP, mmHg	77.7 (10.9)	77.6 (11.4)	78.21 (9.0)	0.97

Data are expressed as mean (standard deviation), median [IQR] or percentages.

Abbreviations: BMI, body mass index; AHI, apnoea-hypopnoea index; ESS, Epworth sleepiness score; ODI, 3% oxygen desaturation index, SBP, office systolic blood pressure; DBP, office diastolic blood pressure.

Table E2: Impact of effective mandibular advancement device (MAD) *versus* sham device on daytime polysomnographic indices

	Effective MAD		Sham device		Adjusted intergroup differences *
	Baseline	Follow-up	Baseline	Follow-up	Mean (95%CI)
AHI, n	40.0 [34.0-51.0]	17.5 † [11.5-25.0]	44.5 [35.0;56.0]	38.5 † [19.0;51.0]	-17.2 (-23.6;-10.8) ll
ODI, n	30.2 (18.5)	15.2 (10.8) †	33.2 (15.6)	28.0 (17.4)	-11.9 (-17.6;-6.1) ll
TST, min	402.7 (74.4)	397.9 (61.9)	376.2 (67.8)	372.3 (71.2)	14.9 (-11.2;41.0)
N1sleep, min	40.9 (32.8)	32.4 (24.5)	36.6 (30.1)	29.6 (21.8)	0.2 (-7.4;7.8)
N2 sleep, min	200.3 (67.3)	194.9 (46.6)	202.0 (69.9)	196.5 (58.7)	-2.3 (-22.6;18.0)
N3 sleep, min	69.5 (45.7)	78.1 (38.7)	61.4 (43.0)	65.8 (40.7)	11.4 (-2.1;24.9)
REM, min	87.0 (30.7)	91.8 (28.6)	68.2 (36.0)	77.3 (34.7) §	6.0 (-6.3;18.2)
MAI, n	32.7 (16.9)	23.1 (11.9) ‡	36.3 (12.8)	31.9 (13.9) §	-7.2 (-12.5;-1.8) **

Data are expressed as mean (standard deviation), median [interquartile range] or mean (95% confidence interval [CI])
Abbreviations: AHI, apnoea-hypopnoea index; AI, apnoea index; ODI, 3% oxygen desaturation index; TST, total sleep time; REM, rapid eye movement sleep; MAI, micro-arousal index.
* Adjusted for baseline value, age, gender and body mass index
† $p<0.001$ *versus* baseline; ‡ $p<0.01$ *versus* baseline; § $p<0.05$ *versus* baseline
ll $p<0.001$; ** $p<0.01$

E References

1 Gagnadoux F, Pépin J-L, Vielle B, *et al.* Impact of Mandibular Advancement Therapy on Endothelial Function in Severe Obstructive Sleep Apnea. *Am J Respir Crit Care Med* 2017;195:1244–52.

2 Gotsopoulos H, Kelly JJ, Cistulli PA. Oral appliance therapy reduces blood pressure in obstructive sleep apnea: a randomized, controlled trial. *Sleep* 2004;27:934–41.

Figure Legends

Figure E1: The effective mandibular advancement device used in the study. Full-coverage acrylic appliances designed to fit onto the upper and lower dental arches are connected by acrylic plates of various sizes allowing adjustment of mandibular advancement. The microsensor thermometer was sealed into the upper arch of the device. The sham device consisted of the upper appliance alone