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Effects of continuous positive airway pressure on endothelial function and circulating progenitor cells in obstructive sleep apnoea: A randomised sham-controlled study ☆

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ABSTRACT

Objective: Obstructive sleep apnoea (OSA) is characterised by reoccurring apnoeas and hypopneas, causing repetitive hypoxia and reoxygenation, and is associated with endothelial dysfunction and reduced levels of circulating progenitor cells (CPCs). The potential to improve endothelial function and CPC levels in people with OSA by preventing hypoxic episodes with Continuous Positive Airway Pressure (CPAP) was investigated in a sham-controlled CPAP study.

Methods: Men with moderate-to-severe OSA (mean ± SD: age = 49 ± 12 y, apnoea hypopnea index (AHI) = 37.6 ± 16.4 events/h, body mass index = 31.5 ± 5.7 kg/m^2) who were CPAP naïve without diabetes mellitus were randomised in a 12-week double-blind sham-controlled parallel group study to receive either active (n = 25) or sham (n = 21) CPAP. CPCs, isolated from blood, were measured by flow cytometry and by co-staining cultured cells (7 days) with acetylated low-density lipoprotein (acLDL) and lectin. Endothelial function was assessed by peripheral arterial tonometry (PAT).

Results: Compared to sham, CPAP significantly decreased AHI (mean between-group difference −36.0 events/h; 95%CI, −49.7 to −22.3, p<0.0001) after 12 weeks. Despite this improvement in AHI, CPAP had no effect on change in CPC levels (including CD34$^+$/KDR$^+$ (565 cells/mL; −977 to 2106, p = 0.45), CD34$^+$/KDR$^+$/CD45$^-$ (37.0 cells/mL; −17.7 to 85.7, p = 0.13), acLDL$^+$/lectin$^+$ (−43.1 cells/field, −247 to 161, p = 0.67)) or change in endothelial function (0.27; −0.14 to 0.67, p = 0.19) compared to sham therapy.

Conclusions: Despite the improvement in OSA parameters and ablation of apnoeic events by CPAP, CPC counts and endothelial function in men with moderate-to-severe OSA were not significantly improved after 12 weeks of therapeutic CPAP when compared to sham control.

1. Introduction

Obstructive sleep apnoea (OSA) is a common disorder that affects up to 25% of adult men and untreated severe OSA is an independent risk factor for all-cause and cardiovascular mortality [1]. However, the direct causal link between OSA and cardiovascular disease (CVD) remains unproven due, in part, to correlating confounding factors such as obesity and a paucity of randomised sham-controlled intervention trials [2]. In OSA, the repetitive partial or complete collapse of the pharynx obstructs the airway, resulting in apnoeas and hypopneas and, consequently, hypoxemia. The intermittent hypoxia caused by OSA is mechanistically implicated in the progression of CVD [3,4], similar to ischemia/reperfusion injury. A marker for subclinical CVD is endothelial dysfunction [5] and, consistent with this, individuals with OSA have been shown to have impaired macro- [6] and microvascular [7] endothelial function compared to healthy controls.

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Circulating mononuclear cells putatively called "endothelial progenitor cells" are intrinsic to vascular repair and regeneration [8–10]. Variously defined either by flow cytometry using all or part of a surface antigen definition of vascular endothelial growth factor receptor 2 (kinase insert domain receptor, KDR)$^+$, CD34$^+$, CD133$^+$ and CD45$^-$, or by culturing ex vivo (defined by Asahara et al. in 1997 [11]), the exact identity of these different cells remains debated [12], however the relevance of these circulating progenitor cells (CPCs) to vascular disease is well established [13]. Cultured CPCs have been found to correlate negatively with Framingham cardiovascular risk score [14] and, in patients with coronary artery disease, the levels of circulating CD34$^+$/KDR$^+$ cells track inversely with future cardiovascular events [15,16]. Both cultured and antigen-defined CPCs have been found to correlate with endothelial function [14,17,18].

Long-term observational studies have shown that Continuous Positive Airway Pressure (CPAP) treatment of OSA is associated with a reduction in fatal and nonfatal cardiovascular events [19,20], but the capacity of CPAP for treating the various vascular pathologies associated with OSA remains to be fully investigated. Sham-CPAP devices [21] modified to deliver sub-therapeutic pressures (<1 cm H_2O) provide a method for conducting double-blinded randomised controlled studies to assess the efficacy of CPAP in treating the disease milieu of OSA.

Here we present the results of a randomised, double-blind, sham-controlled, parallel group study investigating the effect of CPAP treatment on arteriole endothelial function, by peripheral arterial tonometry (PAT), and both antigen-defined and cultured CPC populations in a subset of 46 consecutive patients from a larger study of 65 men with OSA [22].

2. Methods

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

3. Trial design

The trial design for this study has been previously described in full [22].

3.1. Setting and participants

Participants were recruited from tertiary sleep clinics at Royal Prince Alfred Hospital and the Woolcock Institute of Medical Research, Sydney, Australia. Eligible participants were CPAP naïve adult men aged ≥18 years, with moderate-to-severe OSA (apnoea hypopnea index (AHI) ≥20 events/h, oxygen desaturation index 3% by pulse oximetry (ODI) ≥15 events/h) as measured by in-laboratory polysomnography. Exclusion criteria was; diagnosed type II diabetes mellitus, minimum oxygen saturation <65% or an AHI >80 events/h or required immediate CPAP treatment, uncontrolled concurrent medical or psychiatric illness, irregular sleep patterns such as shift-workers, or contraindication to CPAP therapy. Participants were also excluded if they had previously used CPAP or if they had participated in another clinical trial in the previous 30 days.

3.2. Design overview

This was a randomised, double-blind, sham-controlled, parallel group study. Following baseline data collection, eligible participants were assigned a unique sequential number in chronological order and were randomised to receive either real or sham CPAP for 12 weeks in a 1:1 ratio. A computer-programme produced randomised permuted blocks with a block size of four. Machine preparation and pressure determination were performed by a person separate to the study investigators. The study investigators were blinded to treatment allocation for the duration of the study.

The study complied with Good Clinical Practice guidelines, applicable regulatory requirements and the Declaration of Helsinki. All participants provided written informed consent to participate in the study, which was approved by the Sydney South West Area Health Service Human Research and Ethics Committee (RPAH Zone). The study is registered with the Australia New Zealand Clinical Trials Network, www.anzctr.com.au, number ACTRN12608000301369.

3.3. CPAP machines and titration

The real and sham CPAP machines (Remstar Auto, Phillips Respironics, USA) were identical in manufacture and appearance to each other and have been used previously at our centre [23] and by others [24]. The sham device delivered airflow with minimal pressure (0.5 cm H_20). Once randomised, each participant underwent a multiple-night home auto-titrating (CPAP group) or imitation (sham group) pressure determination study. Usage of ≥4 h for at least one night was required before the pressure determination study was accepted. Average mask leak of <0.4 l/s with a mean AHI of ≤10 events/h was required in real CPAP users before a pressure could be determined. Objective compliance data were downloaded from all real and sham CPAP machines after the home titration and at each visit.

4. Polysomnography

Sleep and breathing were assessed by attended overnight, in-laboratory polysomnography (PSG, Sandman Elite v9.2, Tyco Healthcare, Denver, Colorado). A standard diagnostic PSG was performed at week 0. At week 12, standard PSGs were recorded while the participant used the randomly allocated (real or sham) CPAP machine. Sleep staging and respiratory events were scored blinded to treatment allocation, using standard contemporaneous criteria [25,26].

5. Endothelial function

Endothelium-dependent vasodilator capacity of arterioles was measured non-invasively using PAT (EndoPAT, Itamar Medical Ltd., Caesarea, Israel) to assess reactive hyperemia index (RHI). Participants lay down in a darkened quiet room with probes mounted on both index fingers and a blood pressure cuff around the right arm above the elbow for the hyperemia testing. The left finger was used as the control. Finger arterial pulse wave amplitude was recorded throughout the protocol which consisted of 3 stages each of 5 minute duration: 1) baseline recording; 2) occlusion of the brachial artery by inflating the blood pressure cuff to 50 mm Hg above the baseline systolic pressure and 3) post-occlusion recording after deflation of the cuff to measure of the generated reactive hyperemia response. RHI is calculated as a ratio of the average amplitude of the PAT signal post-to-pre occlusion of the tested arm, normalised to the concurrent signal from the contralateral finger.

6. CPC determination

Fasting venous blood samples (30–40 mL) were collected the morning after PSGs at baseline and at week 12 into vacuum tubes containing ethylenediamine tetra-acetic acid (EDTA) as anticoagulant. Within 30 min of collection, peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation (Lymphoprep, Axis Shield, Scotland). CPC populations within the PBMCs were determined by flow cytometry and cell culture.

6.1. Flow cytometry of CPCs

PBMCs were stained with anti-human monoclonal antibodies: fluoresceine-isothiocyanate-conjugated anti-CD34 (CD34-FITC), phycoerythrin-conjugated anti-KDR (KDR-PE) and PE-Cy5-conjugated anti-CD45 (CD45-PC5). Fluorophore-conjugated IgGs were used as isotype controls. For each sample, 500 000 events were acquired and