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The anti-IL-17A antibody secukinumab does not attenuate ozone-induced airway neutrophilia in healthy volunteers

To the Editor:

Within the lung the T-helper cell interleukin (IL)-17 (also referred to as IL-17A) is recognised as one of the principal cytokines that links the activation of T-lymphocytes to the chronic accumulation of neutrophils. It has been proposed that IL-17A is a relevant pharmacological target in inflammatory lung diseases with sustained mobilisation of neutrophils [1].

Secukinumab (AIN457; Novartis Healthcare Pvt. Ltd, Basel, Switzerland) is a recombinant high-affinity fully human monoclonal IL-17A antibody of the immunoglobulin (Ig)G1κ isotype, which binds to human IL-17A and neutralises the bioactivity of this cytokine [2].

Ozone exposure in healthy volunteers induces an acute and reproducible neutrophilic airway inflammation, which can be utilised as a pharmacological model to test anti-inflammatory drugs in early development [3–5].

We performed a single centre, phase II, double-blind, placebo-controlled, parallel-group study with an open label reference arm, investigating the ability of secukinumab to attenuate airway neutrophilia in induced sputum 24 h and 48 h following ozone exposure in healthy volunteers. Subjects underwent a 3 h ozone challenge, and induced sputum samples were collected 24 h and 48 h after the challenge. Subjects with an inflammatory response to ozone (as reflected by an increase in the total number of sputum neutrophils by at least 50% compared with the screening visit) were then randomised to receive either secukinumab (10 $mg \cdot kg^{-1}$ bodyweight) or placebo, in a double blind fashion administered as an infusion over 120 mins or to an open-label single-dose oral corticosteroid treatment (OCS, 50 mg) in the ratio of 2:1:1 (secukinumab:placebo:OCS).

The secukinumab/placebo treatment period consisted of a single dosing visit on day 8 followed by an ozone challenge 1 week later on day 15 and subsequent sputum inductions on day 16 and day 17. A third ozone challenge was performed on day 36 in the first nine subjects and on day 64 in the second nine subjects assigned to secukinumab or placebo. Subjects assigned to OCS treatment received a single dose treatment 1 h before the start of the ozone challenge on day 15. Sputum induction followed 24 h and 48 h after the challenge.

The Ethical Committee of Schleswig-Holstein, Germany approved the study and all subjects provided written informed consent.

The inhalation of 250 ppb ozone and processing of sputum was performed as previously described [3–5].

Secukinumab concentrations in serum were determined by a competitive ELISA assay. Safety assessments and an assessment of immunogenicity to secukinumab were performed at various visits.

The study tested the null hypothesis of no difference between the secukinumab and placebo group in total number of neutrophils in induced sputum 24 h after ozone. *Post hoc* power assumptions using the observed standard deviation of $4.2 \times 10^6 \cdot mL^{-1}$ showed that 12 and 6 subjects in the secukinumab and placebo groups, respectively, provide 80% power to detect a difference of $5.5 \times 10^6 \cdot mL^{-1}$ using a two-sided test at 10% alpha. Standard ANCOVA methods, including baseline adjustment, were applied to neutrophil counts. Data were log-transformed to achieve a normal distribution. No alpha adjustments were made.

24 Caucasian subjects (19 males and five female with mean: age 40 yrs and body mass index 24.2 $kg \cdot m^{-2}$) were enrolled in the study, of which 23 subjects completed the study. One subject was withdrawn for administrative reasons. The mean sputum neutrophil count at screening was $0.63 \times 10^{6} \cdot mL^{-1}$ and increased to $4.19 \times 10^{6} \cdot mL^{-1}$ after the baseline ozone challenge.

Regarding the primary end-point in this study, there was no significant difference observed between the treatment groups in the change from baseline in the total number of sputum neutrophils 24 h after the day 15 ozone challenge (day 16) (fig. 1). Sputum neutrophil counts were higher in secukinumab- and OCS-treated subjects when compared with those receiving placebo 48 h after day 15 ozone challenge (day 17), with the latter reaching statistical significance. There was no significant effect of either secukinumab or placebo on the change from baseline in total number of neutrophils in induced sputum 24 h or 48 h after day 36 or day 64 challenges, with the exception of secukinumab-treated subjects 48 h after the day 64 challenge; driven by an unusually high number of sputum neutrophils in the placebo group. Evaluation of the change from baseline in the per cent of neutrophils did not reveal any treatment effects at any of the time points (data not shown).

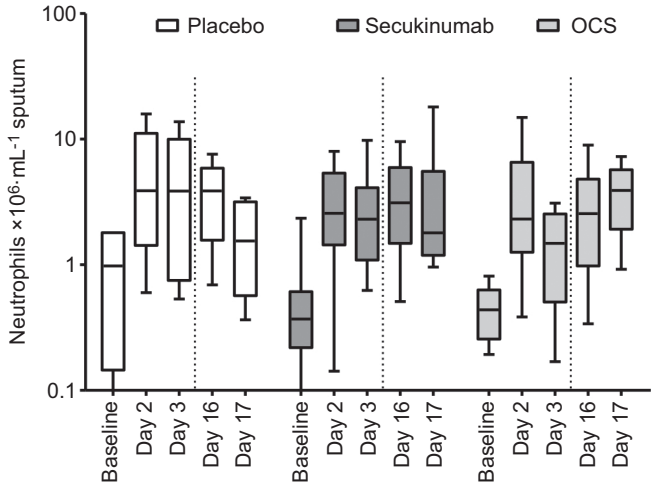

FIGURE 1. Box plot of the total number of sputum neutrophils. Sputum was induced at screening (baseline), 24 h and 48 h after pretreatment ozone challenge (days 2 and 3, respectively), and 24 h and 48 h after post-treatment ozone challenge (days 16 and 17, respectively). The box represents the upper and lower quartiles, the horizontal line the median, and the whiskers the minimum and maximum values. The dashed line represents the timing of treatment (day 8 for placebo and secukinumab subjects and 1 h before ozone challenge on day 15 for oral corticosteroid (OCS) subjects).

The mean exposure as measured by area under the curve extrapolated to infinity ($AUC_{0–inf}$) was 6,414 $day \cdot \mu g^{-1} \cdot mL^{-1}$ with a maximum concentration (C_{max}) of 255 $\mu g \cdot mL^{-1}$ was achieved at a median time of 2.15 h after the start of infusion. The mean half-life was 29.8 days. Serum concentrations of secukinumab declined rapidly until day 7 and then reduced more slowly over the subsequent days.

No unexpected events were observed and no subject had a serious adverse event. The overall incidence of the generally mild adverse events was similar in the secukinumab group (n=8; 67%) and placebo group (n=4; 67%). There were no clinically relevant changes in laboratory tests, ECGs, vital signs, spirometry or pulse oximetry, although a 97% (mean difference $3.25 \times 10^6 \cdot mL^{-1}$) increase in blood neutrophils was noted 24 h after dosing in the OCS-treated group (no increase was observed in the other groups). No immunogenicity was observed in any of the subjects in this study.

We could not demonstrate a treatment effect of secukinumab on ozone-induced airway neutrophilia in this study. There are several potential explanations for these negative findings. It is possible that a higher dose of secukinumab might be required to fully neutralise local IL-17A production following ozone exposure. However, the dose used in this study was based on preclinical modelling and the doses currently used in other inflammatory disorders like psoriasis and rheumatoid arthritis [2].

Another possibility is that IL-17A inhibition alone is not sufficient to inhibit ozone-induced airway neutrophilia in humans. Repeated ozone exposure [6] and lipopolysaccharide exposure [7] in mice has been demonstrated to result in IL-17A dependent airway inflammation. The ozone model was chosen primarily due to the safety of the model and the non-invasive character of sputum induction. Nevertheless it cannot be ruled out that using, *e.g.* segmental endotoxin challenge, an inflammatory stimulus, which mimics bacterial infection and is associated with an IL-17A response in humans [8], would have yielded different results.

A comment is warranted regarding the non-effectiveness of OCS in the control group. We recently demonstrated an acute anti-inflammatory effect of OCS on sputum neutrophils 3 h after the end of the ozone challenge [3, 4]. In the current study, unlike our previous studies, we studied induced sputum 24 h after the start of the ozone challenge and not 3 h post ozone challenge, based on nonclinical data suggesting that IL-17A is involved in a delayed (24 h) response to lipopolysaccharide [7]. The lack of attenuation in sputum neutrophils by OCS treatment may be related to a systemic neutrophilic leukocytosis 24 h after administration [9], potentially inducing a relative increase in the number of leukocytes in other compartments, such as the airway [10]. This assumption is supported by the observation that blood neutrophil counts in the six subjects receiving OCS increased by a mean of 97%, 24 h after dosing and ozone challenge, whereas the blood neutrophil counts in the other treatment groups were stable following ozone challenge.

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Cough frequency in health and disease

To the Editor:

Chronic cough is a difficult clinical problem, partly because there is an absence of well-validated means to assess cough [1, 2]. We have previously reported that the semi-automated computerised Leicester Cough Monitor detects cough accurately over 6 h and that cough frequency is increased in patients with chronic cough compared to controls [3, 4]. There remains uncertainty on the performance of the system over 24 h and across the range of expected cough frequency in larger populations. We set out to address these questions in healthy adult volunteers and adult volunteers with respiratory disease.

44 healthy volunteers were recruited from those responding to a poster advertisement. All reported no current respiratory symptoms, were nonsmokers with a <5 pack-yr past smoking history and had normal spirometric values, methacholine airway responsiveness and induced sputum inflammatory cell counts. 78 patients with respiratory disease were recruited from respiratory clinics. The diagnostic criteria for the conditions have been described previously [5]. Six current smokers and four males taking angiotensin converting enzyme inhibitors (ACEi) were also recruited. The study was approved by the Leicestershire, Northampton and Rutland Research Ethics Committee.

All volunteers underwent spirometry and those with normal spirometry had a methacholine inhalation test using the tidal breathing method. The Leicester Cough Monitor (iRiver iFP-799 mp3 device; iRiver Europe GmbH, Eschborn, Germany and Sennheiser MKE 2–5 field microphone; Sennheiser electronic GmbH & Co. KG, Wedemark, Germany) was attached and recordings obtained and analysed as previously described [6].

The validity of the Leicester cough algorithm was assessed by randomly selecting 20 recordings (eight healthy volunteers, 12 patients with respiratory disease) which were analysed manually [3] by a blinded observer and then by the Leicester cough algorithm. The patients with respiratory disease had the following diagnoses: unexplained chronic cough (n=6), asthma (n=3), eosinophilic bronchitis (n=2) and chronic obstructive pulmonary disease (n=1). A capsaicin cough challenge was performed after removal of the cough monitor [5]. Sputum was induced and processed as described previously [7]. Patients with respiratory diseases completed the Leicester Cough Questionnaire (LCQ) [8]. Patients also completed a 100-mm cough Visual Analogue Score (VAS) [3].

Coughs per 24 h, C2 and C5 (concentration of capsaicin required to elicit two and five coughs, respectively) were log normally distributed and were log transformed prior to analysis. An unpaired t-test was used to compare means between groups. Agreement was assessed by the intra-class correlation coefficient. Correlations between variables were analysed using the Pearson correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non-parametric data. Statistical analysis was performed using SPSS (version 16; SPSS Inc., Chicago, IL, USA).

The geometric mean (log$_{SD}$) manual and Leicester cough algorithm counts per 24 h in 12 patients with conditions associated with cough and eight healthy volunteers was 467 (0.32) *versus* 446 (0.32) and 16 (0.7) *versus* 22 (0.4), respectively. There was stronger agreement as assessed by the intra-class correlation coefficient between manual and counts in patients (0.98) than in healthy volunteers (0.85). The sensitivity and specificity, respectively, of the automated system was 83.8% and 99.9% in patients and 82.3% and 99.9% in healthy volunteers.

In the total population of healthy adults the geometric mean (log$_{SD}$) number of coughs per 24 h was 18.6 (0.5). Females coughed more than males (geometric mean (SD) 29.5 (0.4) *versus* 8.3 (0.5); mean difference 3.5-fold; 95% CI 1.9–6.8; $p<0.001$). There was no correlation between 24-h cough frequency and C2 ($r= -0.08$) or C5 ($r= -0.03$). Daytime cough counts (08:00–22:00 h) were higher than night-time cough counts and hourly cough frequency tended to be higher in early morning and mid-afternoon. Cough frequency was not related to body mass index ($r=0.08$) or age ($r=0.12$).