Neutrophil extracellular traps and CXCR2 antagonism in chronic obstructive pulmonary disease: A pilot randomized control study

Keir HR1, Richardson H1, Mayhew D1, Waite SI1, Fillmore C2, Lazaar AL3, Miller BE4, Tal-Singer R5, Chalmers JD1, Mohan D1

1. Ninewells Clinical Research Centre, University of Dundee, Dundee, UK 2. GSK R&D, Collegeville, PA, USA 3. GSK R&D Stevenage & Stockley Park, UK


Background

- Neutrophil extracellular traps (NETs) are implicated in exacerbations, microbiome dysbiosis and disease progression in patients with chronic obstructive pulmonary disease (COPD).
- IL-8 has been shown to induce NET formation via activation of CXC chemokine receptor 2 (CXCR2).
- In vitro studies have suggested that a CXCR2 antagonist may reduce NET formation induced by COPD sputum.
- Danirixin is a competitive CXCR2 antagonist which has been shown potent antagonist of CXCR2 in vitro.

Aim: We conducted a pilot study to understand whether 14 days of treatment with danirixin leads to a reduction in NET formation and a change in the lung microbiome of COPD patients.

Methods

- Participants were enrolled in a double-blind, randomized, placebo controlled single centre study (NCT03250689).
- Included criteria:
  - Clinical diagnosis of COPD (FEV1 >40% predicted and FEV1/FVC <0.7)
  - Elevated sputum NET levels (>0.5 units/mL) at screening.
- Participants were randomized 3:1 to receive either danirixin 35mg or placebo for 14 days.
- Key assessments included spirometry and induced sputum at screening, day 1, 7 & 14.
- Sputum measurements included:
  - NETs by immunoassay (histone-elastase and DNA-elastase complexes)
  - NETs by confocal microscopy
  - Microbiome by 16S rRNA gene sequencing
  - Sputum neutrophil count
  - Sputum resistin and neutrophil elastase by ELISA.

Conclusion

- Measurement of NETs is feasible in a clinical trial.
- Study results showed no evidence that blocking CXCR2 activation via danirixin reduced NET formation or changed the lung microbiome composition in patients with COPD.
- Subsequent in vitro studies demonstrated that danirixin inhibits neutrophil activation in response to CXCR2 ligands but at the doses used some residual activation was observed.
- These results suggest that there is a subset of patients with COPD in whom NET formation is independent of IL-8.

Results 1

43 participants were screened, with 19 randomised (14 danirixin; 5 placebo), out of a planned 32. The study was terminated early due to cessation of the danirixin development program.

- There was no significant difference in percentage change from baseline in NETs between the danirixin and placebo groups (Fig. 1) as measured by:
  - Histone-elastase complexes, the primary endpoint, (mean change +3.2% [SE 22.3] vs -24.7% [16.9]). Data in Fig 1 represent median histone-elastase complexes across the trial visits, from a post hoc analysis, whereas the primary analysis was from the mixed model
- DNA-elastase complexes (mean +33.9% [SE 56.7] vs 29.3% [3.7]).
- Confocal microscopy of NET area per cell (mean 30.8% [SE 42.9] vs 103.1% [106.1])
- There was no change from baseline in sputum neutrophil count between danirixin (-14.9% [SE 17.34]) and placebo (-7.6% [SE 5.33]).
- ELISA measurement of neutrophil biomarkers, neutrophil elastase and resistin, in sputum saw no difference between the danirixin and placebo groups.

Results 2

- Analysis of the airway microbiome showed that there were no significant differences between treatment groups in overall microbiota composition (Fig. 2A), alpha or beta diversity, total bacterial load or relative Haemophilus abundance.

In vitro assays: Investigating the effects of danirixin on neutrophil activation

- As part of a separate GSK-University of Dundee collaboration, we carried out a series of in vitro experiments to examine whether neutrophil activation is IL-8 dependent in COPD since sputum IL-8 levels are heterogeneous in patients with COPD.
- Neutrophils from healthy donors treated with IL-8 (10 ng/mL) (Fig. 3A) or GRO-alpha (1000 nM), a specific CXCR2 agonist, (Fig. 3B) showed a significant increase in surface CD11b expression which was blocked by danirixin pre-treatment.

Microbiome

- Analysis of the airway microbiome showed that there were no significant differences between treatment groups in overall microbiota composition (Fig. 2A), alpha or beta diversity, total bacterial load or relative Haemophilus abundance.

References


Disclosures

- GSK study 207551 was funded by GSK (NCT03250689). The in vitro assays were part of a separate GSK-University of Dundee collaboration where GSK only provided danirixin investigational product.
- HK has no conflict of interest to declare, DM is a current employee and shareholder of GSK. Editorial support (in the form of editorial alignment with congress guidance and collating author comments) was provided by Kirsty Millar, MSc, of Gardiner-Caldwell Communications (Macclesfield, UK), and was funded by GlaxoSmithKline plc.