ARTICLE DETAILS

TITLE (PROVISIONAL)  Sputum PGP is reduced by azithromycin treatment in COPD patient and correlates with exacerbations

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VERSION 1 - REVIEW

REVIEWER  Farrah Kheradmand
Baylor College of Medicine

REVIEW RETURNED  25-Oct-2013

GENERAL COMMENTS  The paper by O’Reilly et al., examines the production and association of PGP peptides in sputum samples collected as part of an ancillary study to examine the efficacy of azithromycin for prevention of COPD exacerbation. They show that compared to placebo, COPD patients treated with Azithromycin have significantly reduced sputum PGP and myeloperoxidase and these findings remained robust and even increased over duration of treatment. Interestingly azithromycin did not change measured levels of MMP9 or increase in de novo PGP generation in sputum samples. Finally they find that PGP measurements peaked around the time of exacerbation and declined following medical intervention.

Overall the data are very clear, informative, and the information provides a possible novel and non-invasive assay to measure PGP as a biomarker of COPD exacerbation. Although number of participants in this study was relatively limited, the authors were able to find meaningful and statistically significant result by comparing data collected in sputum of azithromycin vs placebo treated group.

A few minor clarifications are listed below:

1- Could the authors provide the clinical information (e.g. FEV1, age, gender, GOLD stage etc.) of the study participants?
2- Did the authors have any information regarding smoking status in this ancillary cohort? If so, did active smoking play a role in differences found in PGP measurement?
3- Data shown in Figure 3 is very interesting but is unclear whether samples are from the placebo or azithromycin treated group; could the authors comment whether the peak in PGP detection could have been affected by azithromycin treatment?
4- Although it may not be feasible, but if the authors have access to other samples, could PGP clearance be detected in other samples (e.g. urine or serum?)

REVIEWER  Robert Snelgrove
Imperial College London,
THE STUDY

Supplementary reporting of trial registration etc is not included in this manuscript but the reported experiments are an ancillary study to an already published trial detailing these criteria.

GENERAL COMMENTS

The neutrophil chemoattractant PGP has been implicated in the pathologies of chronic lung diseases such as COPD but evidence has thus far been lacking. Similarly, it is unknown whether PGP levels correlate with severity of COPD and can act as a predictor/biomarker and whether PGP is treatment sensitive. This study is therefore timely and intriguing in addressing these objectives, with studies appropriately conducted and discussed. There are a few minor comments:

- A total of 46 sputum samples were obtained from 21 patients with samples taken at various time points from some patients. However, data is presented indiscriminately of patient donor. Is it possible for the authors to comment, given the limited data size, on how PGP sputum levels change within specific individual patients who have samples collected at various points; i.e. do all patients show a relative reduction in PGP with length of azithromycin treatment and do all patients show a relative spike in PGP levels around time of exacerbation.

- How do MPO levels track with exacerbation and do MPO levels correlate well with PGP levels (both placebo vs azithromycin and exacerbation data)? As such are PGP levels a good predictor of neutrophilic inflammation? Do the authors believe this would reflect the PGP causing the neutrophilic inflammation or being a consequence of the neutrophilic inflammation (since neutrophils possess PGP generating enzymes)?

- In the inset of figure 3, on what basis were the boundaries for pre-, peri- and post- exacerbation determined, e.g. on what criteria was it decided to classify peri- as 18+-/- 4 days either side of an exacerbation.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1:

1. Clinical characteristics of our study subjects were similar to those of the parent trial: age = 67.6 ± 8.4 years, 30% female, post-bronchodilator FEV1 = 1.36 ± 0.47L (mean ± SD). The manuscript has been changed to include this information (Results, part 1).

2. Only six of the 21 subjects in our study were current smokers and there was no significant effect detected of smoking on PGP levels.

3. Data on PGP levels around the time of an exacerbation came from both placebo and azithromycin-treated groups and the manuscript has been changed to clarify this (Results, part 3). There was no apparent effect of azithromycin treatment on PGP levels during exacerbations.

4. We can measure PGP in both urine and serum. Although these samples were not studied by us in this ancillary study, they could be included in future clinical studies of COPD.
Reviewer 2:

1. Given the limited data set, effects of azithromycin treatment on PGP sputum levels were reported for groups. However, there was a progressive decline in sputum PGP with duration of treatment in 5 of 6 azithromycin-treated subjects whose sputum was assayed at more than one time-point. The manuscript has been changed to include this information (Results, part 1). The data on PGP levels around the time of an exacerbation reflect all the exacerbations experienced by our subjects during the study period; they are insufficient to show if all subjects show a similar spike in PGP as not all subjects experienced an exacerbation and sequential sputum samples were not available for all subjects.

2. Although sputum MPO levels declined with azithromycin treatment along with PGP (albeit to a lesser extent), we found a weak and non-significant relationship between PGP and MPO levels in individual sputum samples. Surprisingly, there was no relationship between sputum MPO and time of exacerbations. This may mean that PGP is a better biomarker for exacerbations. We believe that PGP feeds forward to worsen neutrophilic inflammation. However, PGP levels may be affected by factors other than neutrophil burden such as breakdown by aminopeptidases, including leukotriene A4 hydrolase.

3. The boundaries for pre, peri and post exacerbation PGP levels were determined by our sputum data and dates of collection of samples. We found that sputum PGP became elevated as long as a month pre exacerbation and remained high as long as a month afterward without completely returning to pre exacerbation levels. The boundaries reported represent the mean ± SEM of these times.

Response to Thorax reviewers:

Reviewer 1:

1. Information regarding the clinical characteristics of our subjects and their similarity to participants in the parent trial has been provided above and the manuscript has been changed to include this information (see response to BMJ Open Reviewer 1). The subjects in our study also showed a similar response to azithromycin as did the the participants in the parent trial, namely a reduction in exacerbations (see Results, part 2).

2. The inter and intra-assay variability of the PGP assay in sputum samples has been reported before (Respir Res. 2009 May 18;10:38. O’Reilly et al). In this study, PGP concentrations in sputum were estimated using a standard curve of known PGP concentrations run concurrently. Spiking experiments were not performed.

4. We agree with the reviewer that an ROC curve would be the ideal way to demonstrate the sensitivity/specificity of sputum PGP to predict exacerbations but this would require a larger trial with increased number of subjects and sequential sputum samples.

Reviewer 2:

1. Although 1142 participants were randomized to the Macrolide trial, sputum samples were collected from only a minority of participants and only some of these samples were made available to us for this ancillary study. Sputum collection was not mandated as part of the parent trial and participation of other centers in our ancillary study was on a voluntary basis.

2. Sputa in the Macrolide trial were produced spontaneously and were not induced. A larger trial using sputum induction would be required to test if our results only pertain to COPD patients who spontaneously produce sputum. However, this does not preclude the potential of sputum PGP to be a biomarker for response to therapy and exacerbations in COPD.

3. We agree with the reviewer that the small sample size in this study makes generalizability an issue. However, we believe that a number of factors mitigate this problem. Firstly, our study was conducted
ancillary to a large, randomized controlled trial. Our subjects, although small in number, had similar characteristics to the participants in the parent trial and had a similar therapeutic response to azithromycin. Finally, that sputum PGP declined further over time in the azithromycin-treated group and that levels of another biomarker (MPO) also declined, make a biological effect more likely. 4. We agree with the reviewer that longitudinal within-patient data is necessary to definitively show that PGP levels are predictive of or elevated during exacerbations. Nonetheless, we believe that our data in a small number of patients support such an association which should be tested further in a larger trial.

Reviewer 3:

1. The reviewer states that our study may reflect only sputum-producing bronchitic patients who might be more likely to respond to azithromycin. A larger trial using sputum induction would be required to test this idea and, as we have stated in reply to reviewer 2, this would not preclude the usefulness of PGP as a biomarker in this subset of COPD patients.

2. We apologize for the somewhat confusing nature of our study design. A total of 46 sputum samples were obtained from 21 different subjects at months 1, 3, 9 or 12 of the trial period.

3. We agree with the reviewer the dose of azithromycin used in the parent trial could have had an anti-infective or anti-inflammatory effect. However, both effects would be expected to reduce neutrophilic airway inflammation and, thereby, PGP levels.
Sputum PGP is reduced by azithromycin treatment in patients with COPD and correlates with exacerbations

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