Reviewer's report

Title: Y-27632 is associated with corticosteroid-potentiated control of pulmonary remodeling and inflammation in guinea pigs with chronic allergic inflammation

Version: 1  Date: 17 February 2015

Reviewer: Richard Kim

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Pigati and colleagues address the very interesting and clinically relevant topic of combining corticosteroids with adjunctive therapies for the control of chronic allergic inflammation. They demonstrate that in allergic guinea pigs treatment with the corticosteroid dexamethasone or the Rho-kinase inhibitor Y-27632 imparted control over several disease features in the airways and distal parenchyma. Importantly, combined treatment with dexamethasone and Y-27632 further reduced aspects of the remodelling response and the expression of inflammatory markers and oxidative stress in the two lung compartments assessed. The authors propose that treatment with a Rho-kinase inhibitor in the absence, or presence, of corticosteroids could be used to maximise control of several disease features associated with chronic allergic pulmonary inflammation. The experiments are largely convincing and the results are interesting. There are a few points to consider:

Major Compulsory Revisions

1. The statement “Only in the lung tissue of the OVA-RHO and ORC groups was there a reduction in the eosinophil density, compared with the OVA-C group, (P<0.05)” (page 18) should be amended. Data presented in Table 1 (page 51) indicates that the OVA-RHO group has reduced eosinophil density also in the airways compared to OVA-C. OVA-RHO=12.17±1.57 */# compared to OVA-C=16.83±4.34 *.

2. The statement “There was a potentiation of this reduction in the airways when both treatments were used” (page 25) is incorrect. Data and statistics presented in Table 2 (page 52) indicate that there is no further reduction in the volume fraction of actin in the airways in the ORC group (15.30±1.23 *) compared to OVA-RHO or OVA-C (12.16±1.25 * and 19.97±0.94 *, respectively).

3. The statement “The combination of corticosteroid with Y-27632 enhanced the reduction in the volume fraction of collagen and elastic fibers present in the airways” (page 27) should be modified. The data presented in Table 2 (page 52) indicates that the volume fraction of elastic fibres in the airways in the ORC group is not statistically different to that of the OVA-RHO group, suggesting that there was no summative reduction when treatments were combined.
Minor Essential Revisions

4. The statement “We also observed a greater attenuation of TIMP-1-positive cells in the lung parenchyma when both drugs were combined” (page 27). The statistics shown in Table 2 (page 52) indicate that the ORC group is significantly different to OVA and OVA-C, but not OVA-RHO – if appropriate amend the statistics denoted in Table 2 to reflect this statement.

5. The statement “The number of MMP-9-positive cells is shown in Figures 15A and 15B” (page 20) needs to be amended. Please clarify whether the data being referred to is in fact presented in Figure 8 and/or Table 2.

6. Please revise and clarify the statement “The ENO level has been proposed as an indirect marker of the inflammatory response to be used in clinical practice, and correlates with the severity and response to the treatment in asthmatics or in animal models of chronic pulmonary inflammation” (page 24).

7. The statement “Souza et al. investigated the effects of the corticosteroid montelukast …” (page 26) is incorrect. Montelukast is not a corticosteroid, it is a leukotriene receptor antagonist.

8. Please revise and clarify the statement “Likewise, Goto et al. suggested that prednisolone is able to inhibit NF-κB transcription induced by the interaction with the corticoid receptor, resulting in inhibition of Rho regulation induced by IL-13 and TNF-α” (pages 29-30).

9. Typographical errors
   a. “INF-κ” should be IFN-κ (pages 23, 26, 51)
   b. “motelukast” should be montelukast (page 26)
   c. “blecomethasone” should be beclomethasone (page 28)
   d. The legend for Figure 4 is entitled “Figure 4A, 4B, 4C and 4D”. The legend is also missing descriptions of the data presented in Figures 4C and 4D.
   e. Keep abbreviation for NF-κB consistent - interchangeably referred to as NF#B, NF-κB and NF-κB (page 29). The abbreviation for NF-κB should be introduced earlier than paragraph 4 on page 29.
   f. Commas, instead of decimal points, have been used in parts of Table 2: i) OVA-RHO group (Lung Tissue, Elastic Fibres), and ii) Sal group (Lung Tissue, TIMP-1). There is also a random apostrophe in the data for the OVA group (Airway, TIMP-1)

Discretionary Revisions

10. Consider swapping Figures 7 and 8 to make the figures consistent with the chronological presentation of data in the Results section.
11. Consider moving eosinophil (LUNA-stained) tissue photomicrographs from Figure 7 to Figure 5 to make consistent with the data with the data presented in Table 1.

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests