Reviewer’s report

Title: Pharmacologic inhibition of S-nitrosoglutathione reductase protects against experimental asthma via both bronchodilatory and anti-inflammatory activities

Version: 3 Date: 23 July 2013

Reviewer: Tim McMahon

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This study examines the effects of a GSNOR inhibitor on indices of inflammation in mice with asthma, while also examining what plethysmographic responses and biomarker patterns in BAL fluid and blood as a function of drug dose as well as the interval between N6022 dosing and the methacholine challenge in OVA-exposed mice. Essentially N6022 is found to be a potent inhibitor of the adverse plethysmographic changes, the eosinophilia in the lungs, and inflammatory biomarkers profiled. In addition, there is inhibition of NFkB activity, a positive effect on BAL fluid nitrite and plasma cGMP, and inhibition of smooth muscle constrictor changes in vitro in rat tracheal rings exposed to methacholine. The findings are fairly impressive and point to substantial and practical therapeutic potential.

Major concerns

1. There is an unexplained absence of any actual direct airway mechanics measurements together with a lack of discussion of the well-established limitations of the “enhanced pause” (Penh) technique. The closest mention of this is in the Methods section where the authors note that the Penh “correlates with the measurement of airway resistance, impedance, and intrapleural pressure.” This would seem to be an important point given that even the title concludes the compound has bronchodilatory effects.

2. The authors acknowledge an inability to detect SNOs in the BAL fluid of asthmatic mice, but some clarifications are needed. First, what was the detection limit and what assay(s) was/were used in the N30 labs and/or the contractor’s lab? Was there an attempt to measure GSNO or other SNOs in the unexposed mice? Please provide a reference for the statement concerning the dilution-induced dissociation of the inhibitors from the enzyme-substrate complex. Finally the authors refer to the inability of other investigators to detect GSNO in asthmatic BALF. A brief discussion of any difference in methods or other variables would be in order here. If the authors have any data on (G)SNO reductase activity in these mice or their BAL fluid using spiked GSNO, that would be supportive.

3. There appears to be a U-shaped relationship between the GSNO inhibitor dose and the Penh changes, eosinophilia, and changes in several of the biomarkers including IL-10, IL-12p70, IL-17A, in BALF and plasma fibrinogen. Please acknowledge the complex dose-response relationship and discuss as
appropriate. Also, it would appear that the effects on BAL fluid fibrinogen and (at both doses) are significant but this is not so marked (error)?

Minor concerns

1. Please provide a reference for the statement concerning the dilution-induced dissociation of the inhibitors from the enzyme-substrate complex.
2. BAL fluid nitrite, Figure 3, please show values from non-sensitized animals. Likewise, for Figure 4, cGMP.
3. Figure 6, consider going beyond calling this effect “sustained” and noting that it appears to strengthen over the time elapsed between inhibitor dosing and challenge.
4. Bottom page 18 and top page 19. Regarding the statement that the findings suggest iNOS-derived NO is not necessarily responsible for SNO levels in the BALF, this statement requires some more explanation or evidence as currently stated.
5. Even putting aside the Buxco/Penh limitations, it would be more accurate to describe N6022 as preventing bronchoconstriction in response to methacholine in sensitized and, to a lesser extent, non-sensitized mice subjected to methacholine challenge, than to refer to bronchodilatory capacity.
6. In the Figure 1 legend, please state that Figure 1D reports on non-sensitized mice.

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

**Quality of written English:** Acceptable

**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.