Reviewer's report

Title: Characterization of a murine model of monocrotaline pyrrole-induced acute lung injury

Version: 2 Date: 30 June 2008

Reviewer: Pravin B Sehgal

Reviewer's report:

In this MS the authors report on their attempt to overcome what has remained as a major technical problem in the field of experimental pulmonary hypertension work – although a single administration of monocrotaline (MCT) into a rat elicits a robust pulmonary arterial hypertension (PAH) 2-4 weeks later, MCT does not elicit this response in the mouse. The technical issue here is that there are a plethora of genetically mutated mice available but not rats. The existing approach to elicit PAH in the mouse involves hypoxia which is cumbersome. Many in the field have spoken often and long about trying out the active derivative of MCT – the pyrrolic MCTP – in the mouse. The authors have carried out these experiments and the present MS reports on their observations. The negative results obtained thus far – that MCTP does not elicit PAH in the mouse – at least when administered in the manner used by these authors are important to disseminate. That MCTP has acute lung toxicity when administered into the jugular veins of mice is not surprising. Overall, although the MS contains important negative data up to a point, several issues need to be addressed by the authors.

A. Major concerns.

1. As the authors state, because MCTP is known to have a short half-life in aqueous buffers (approximately 3 sec), the exact manner of instillation into the animal is critical. Thus, the comparison between MCT (administered subcutaneously) and MCTP (administered intra jugular vein in DMF as solvent) in producing PAH in the rat is critical in order to validate the technique used (pg. 9 top and Fig. 1). The text on pg 9 states that the two were “comparable”. However, the data in Fig. 1 showing RVH are missing statistical comparison between the MCT and MCTP groups. From the data in Fig. 1 it appears that MCTP as used in this study was not as effective as MCT. This is an issue that must be addressed. Moreover, the text on pg. 9 contains data for RVSP but the Figure does not. Please include RV systolic pressure data in Fig. 1 and do statistical comparisons between the MCT and MCTP groups not just with the DMF controls.

2. That the particular route of administration intrajugular (in the rat or mouse) delivers a single rapid bolus as opposed to sc MCT in the rat which leads to, in essence, a slow constant release of MCTP from the liver over several hours and perhaps the first day, must be discussed as a limitation of this study. Someone in
the future could perhaps use a long-lasting pump device to administer MCTP into the mouse jugular vein slowly over several days and obtain different results. Please discuss (also see item 4 below).

3. Please carry out mass spec analyses to indicate how much of the MCT was converted to MCTP in the samples used in this study. Also please review the text throughout to fill in the exact concentrations of MCTP injected – many places are left as “9 g/kg body weight” (see pg. 4 for one example.

4. A major issue in this MS is that MCTP administration into the jugular vein of mice produces acute lung injury but not the slower developing PAH. Does MCTP cause “megalocytosis” of murine endothelial cells? This is a simple experiment to carry out in cell culture and would serve as an important positive control for the efficacy of MCTP on mouse cells compared to say rat or human or bovine PAECs.

B. Minor issues:

1. Please revisit the lay-out and graphics in all of the line drawing figures. At least in my printed copy, these were not particularly esthetic.

2. Please correct typos on pg. 7 – 4th and 3rd line from bottom.

3. In the future please do not upload a cover letter for Am J Resp Crit Care Med (dated March 21, 2008) when sending the MS to another journal (in this case BMC Pulmonary Medicine).

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests.