Title: Tetrahydrobiopterin improves pulmonary vascular remodeling following mouse-intratraqueal bleomicin administration

Dr. Patricia 2191 Almudever patricia.almudever@uv.es 1, Prof. Dr Ricardo 2192 Guijarro guijarro_ricjor@gva.es MD 2, Dr. Javier 2193 Milara xmilara@hotmail.com 3, Dr. Kaya 2194 Marini marini@yahoo.co.uk 1 and Prof. Dr Julio 2195 Cortijo julio.cortijo@uv.es 4. 1 Pharmacology Department, Valencia University, Valencia, Spain, 46010; 2 Thoracic Surgery Department, Valencia University (General Hospital), Valencia, Spain, 46010; 3 Biothechnology Department, Politecnic University, Valencia, Spain, 46010 and 4 CIBERES, Valencia University, Valencia, Spain, 46010.

Body: Background/Objective: Pulmonary hypertension in pulmonary fibrosis portends a poor prognosis. Recent evidence suggests that tetrahydrobiopterin (BH4), the cofactor of nitric oxide synthase, is involved in pulmonary hypertension. However the role of BH4 in pulmonary hypertension secondary to pulmonary fibrosis is unknown. The current study investigated the role of BH4 on pulmonary remodelling in an animal model of bleomycin-induced lung fibrosis. Methods: C57Bl/6J mice were instilled intratracheally with a single dose of bleomycin at 3.75 U/kg at day 1. BH4 (20mg/kg) or vehicle (control) was administered orally once a day, from day 1 until the end of experiment (day 14). At the end of the treatment period, mice were sacrificed and plasma, lungs and heart were removed. Plasmatic BH4 concentration was measured by high performance liquid chromatography. The right ventricular (RV) wall of the heart was dissected free and weighed along with the left ventricle wall plus septum (LV + S), and the resulting weights were reported as RV/LV + S ratio to provide an index of right ventricular hypertrophy. TGF-β1 and ET-1 gene expression were measured by real time RT-PCR in lung homogenates as pulmonary vascular remodeling markers. Results: Bleomycin reduced ~2.3-fold the BH4 plasmatic levels, augmented the RV/LV + S ratio to 0.075 mg/mg over control, and increased the ET-1 and TGF-β1 gene expression to ~2-fold and ~6-fold versus control respectively. Oral BH4 suppressed the bleomycin-induced right ventricular hypertrophy and reduced the ET-1 and TGF-β1 gene expression to control levels. Conclusions: BH4 inhibits bleomycin-induced right ventricular hypertrophy in mice.