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A key role for acid sphingomyelinase in pulmonary vascular dysfunction induced by lipopolysaccharide.

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Acute Lug Injury (ALI) is characterized by pulmonary edema and alveolar collapse resulting in severe arterial hypoxemia. Although studies defining appropriate ventilator management have improved patient outcomes, the mortality associated with ALI remains unacceptably high. Pulmonary vascular dysfunction is a prominent feature independently associated with poor outcomes in patients with ALI (Bull et al., 2010). Acid sphingomyelinase (SMase) has been postulated to modulate pulmonary edema in experimental ALI (Göggel et al., 2004). Recently, we demonstrated that neutral SMase-derived ceramide is critical in the regulation of pulmonary vascular tone (Cogolludo et al., 2009; Moral-Sanz et al., 2011). The aim of this study was to analyze the role of SMase on lipopolysaccharide (LPS)-induced pulmonary vascular dysfunction.

Male Wistar rats (270-330g) were killed by cervical dislocation and vascular reactivity was assessed using intrapulmonary arteries mounted into isometric wire myographs. Pulmonary artery (PA) rings were incubated for 20 hours in DMEM alone (control) or DMEM containing LPS (1 µg/mL) or bacterial SMase (0.01U/mL; from *Bacillus cereus*). In additional experiments, PA were pre-treated for 45 min with the acid SMase inhibitor D609 (100µM) before LPS was added. Release of NO (via its breakdown product, nitrite using the Griess assay) by pulmonary tissue and IL-6 (Elisa, R&D Systems) by rat pulmonary artery smooth muscle cells (PASMCs) was measured at 20 h.

	IL-6 (ng/mL)	**NITRITE (µM)**	**Phenylefrine E_{MAX} (mN/mm^2)**	**Serotonin E_{MAX} (mN/mm^2)**	**Acetylcholine E_{MAX} (% of relaxation)**
CTRL	0.3±0.2	1.7±1.2	1,8±0,2	2.1±0.3	61±5
LPS	14.9±0.7*	20.3±2.3*	0.9±0.4*	4.3±1*	15±7*
SMase	4.7±0.3*/#	9.1±2.6*	0.9±0.3*	3.3±0.9	35±3*/#
D609+LPS	8.9±0.1*/#	21.3±4.9	0.8±0.4*	2.3±0.9#	53±6#

*Table 1. Role of acid SMase in the effects induced by LPS on pulmonary vascular function. E_{MAX}, Maximal effects induced by the following vasoactive factors in isolated pulmonary arteries: phenylefrine (1nM-10µM), serotonin (1nM-30µM) and acetylcholine (1nM-10µM). The relaxant responses to acetylcholine were tested in arteries pretreated with serotonin (10µM). Data are shown as mean ± S.E.M. of n = 4-12. * and # p<0.05 by one way ANOVA followed by Dunnett's post hoc test compared to control or LPS-treated arteries, respectively.*

Exposure to LPS for 20 hours markedly increased nitrite production by PA rings and IL-6 release by PASMCs. Whereas LPS reduced the ability of vessels to contract in response to phenylphrine, serotonin-induced pulmonary vasoconstriction was significantly increased and the relaxation responses to acetylcholine were markedly reduced (Table 1). Similar changes on pulmonary vascular responses were observed following addition of bacterial SMase. The acid SMase inhibitor D609, prevented LPS-induced hyperresponsiveness to serotonin and endothelial dysfunction. D609 also inhibited LPS-induced IL6 release by rat PASMCs but had no effect on the ability of LPS to induce hyporeactivity to phenylephrine and nitrite production by PA rings.

In conclusion, bacterial SMase induces pulmonary vascular dysfunction mimicking the effects of LPS. In addition, inhibition of acid SMase prevents LPS-induced endothelial dysfunction and hyperresponsiveness to serotonin despite a lack of inhibitory effects on inducible NO synthase (NOSII) activity. These findings suggest that acid SMase may represent a potential therapeutic target for the treatment of pulmonary vascular dysfunction associated to bacterial sepsis.

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