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Time course of nitric oxide synthases and oxidative stress in ovine sepsis

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Background: Previous studies revealed the important roles of different isoforms of nitric oxide synthases (NOS) and determinants of oxidative stress in the pathophysiology of cardiopulmonary derangements in sepsis, thereby offering potentially new treatment options such as inhibition of NOS or inhibition of reactive nitrogen species and poly(ADP-ribose) polymerase. When considering possible treatment strategies, however, it is crucial to identify the time changes of the expression of the pathogenic factors that are involved in the pathophysiology of sepsis. The present study was conducted to determine the time course of endothelial NOS (eNOS), neuronal NOS (nNOS), inducible NOS (iNOS), 3-nitrotyrosine (3-NT), and poly(ADP-ribose) (PAR) in lung tissue using an established model of ovine sepsis.

Methods: Twenty-four sheep were instrumented for chronic study. After inhalation of 48 breaths of cotton smoke, live *Ps. aeruginosa* was instilled into both lungs. Sheep were sacrificed at 4, 8, 12, 18, and 24 h after induction of sepsis (n=4 per group). Additional four sheep received sham injury and were sacrificed after 24 h.

Results: In all sheep, PaO_2/FiO_2 ratio was below 200 at 18 h, indicating acute respiratory distress syndrome (ARDS). Lung wet-to-dry weight ratio, an indicator of lung water content, started to increase 4 h after injury and reached a peak at 24 h. Expression of eNOS was increased between 4 and 18 h after injury ($p<0.05$), reaching a peak at 12 h. Expression of iNOS was increased between 8 and 18 h ($p<0.05$), peaking also at 12 h. These changes were associated with a rise in plasma nitrite/nitrate levels ($p<0.05$). Expression of nNOS did not increase throughout the study. Expression of 3-NT, a stable marker of peroxynitrite, was increased between 4 and 12 h ($p<0.05$), peaking at 8 h. Expression of PAR started to increase 4 h after injury ($p<0.05$), peaking also at 8 h.

Conclusion: The increased expression of eNOS and iNOS was associated with increases in both 3-nitrotyrosine and PAR expression. The time changes of oxidative stress correlated with the early development of ARDS and pulmonary edema in this model.

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Aspirin-triggered lipoxin A4 differentially modulates HO-1 expression and NADPH oxidase activity in endothelial cells: implications in pro-oxidative, proinflammatory processes.

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Lipoxins (LX) and aspirin-triggered LX (ATL) are eicosanoids generated during inflammation via transcellular biosynthetic routes, which have emerged as mediators of key events in endogenous anti-inflammation and resolution. We have reported that an aspirin-triggered lipoxin A_4 analog (ATL-1) induces the expression heme oxygenase 1 (HO-1) in endothelial cells (EC), an inducible enzyme closely related with the resolution phase of inflammation. This process requires de novo protein synthesis via LXA_4 receptor. Moreover, ATL-1-triggered HO-1 expression inhibited VCAM and E-selectin expression induced by proinflammatory cytokines, what may lead to an efficient impairment of leukocyte-endothelium interactions during the onset of inflammation. These data showed, for the first time, the convergence of these two pillars of resolution of inflammation.

More recently, we observed that ATL-1 also impairs ROS generation by EC, a phenomenon closely related to pro-oxidant, proinflammatory and pro-thrombotic cardiovascular conditions, such as hypertension, atherosclerosis, and heart failure. Pre-treatment of EC with ATL-1 (1 - 100 nM) completely blocked ROS production triggered by different agents, as assessed by dihydrorhodamine 123 and hydroethidine. Furthermore, ATL-1 inhibited the phosphorylation and translocation of the cytosplamic NAD(P)H oxidase subunit $p47^{phox}$ to the cell membrane as well as NAD(P)H oxidase activity. ATL-1 treatment also impaired the redox-sensitive activation of the transcription factor NF-kappaB, a critical step in events associated to inflammatory and vascular pathologies. The involvement of distinct protein kinases/phosphatases in this phenomenon are under investigation.

We believe that a better understanding of ATL ability to modulate ROS-sensitive processes, interfering with two pathways closely related to the maintenance of cellular redox homeostasis, may lead to the development of new strategies in order to control the redox imbalance associated with several pathological situations associated with a proinflammatory, pro-oxidative component.

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Oxidative damage is the biochemical mechanism of cellular injury in choline deficiency.

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Oxidative damage is the pathogenic mechanism involved in the development of the lesions induced by choline-deficiency (CD). It produces oxidative damage in liver, heart, kidney and brain by lipid peroxidation, decrease of antioxidants, and consumption of tissue alpha-tocopherol. Weanling Wistar male rats were divided into two groups: one group fed a choline-deficient diet (CD); and the other fed a choline-supplemented diet as control (CS). Liver, heart, brain and both kidneys were removed for oxidative damage determinations: thiobarbituric reactive substances (TBARS), and tert-butyl hydroperoxide initiated chemiluminescence (CL-BOOH), and histopathological analysis. The histopathological study showed: hepatic esteatosis (first day), renal necrosis (fifth day), and heart necrosis (seventh day). In tissues homogenates, TBARS increased by 35 % in liver (third day, $p<0.05$); 80 % in kidney (third day, $p<0.05$); more than 100 % in heart (fifth day, $p<0.01$), and 70% in brain (sixth day, $p<0.05$). Oxidative stress reaches systemic significance in CD since 4-times increased plasma levels of TBARS with a $t_{1/2}$ (the time for half maximal effect) of 2.8 days were observed. The normal rat plasma level (1.5 microM) was increased by 200% at the third day ($p<0.02$), 400% at the fifth day ($p<0.04$) and 300% at the sixth day ($p<0.05$). CL-BOOH was increased at the sixth day by 45% in liver ($p<0.05$), 83% in kidney ($p< 0.01$), 50% in heart and 114% in brain ($p<0.05$ respectively). TBARS is the earliest sign of the pathogenic effect of CD, followed by the increase in CL-BOOH, meaning decreased tissue levels of lipid soluble antioxidants, i.e. endogenous alpha-tocopherol, and by the histopathological damage. In the liver, the morphological damage is previous to the lipid peroxidation and the consumption of endogenous antioxidants. In kidney and heart, indeed, lipid peroxidation and oxidative damage are preceding the necrosis. These results indicate that lipid peroxidation plays a role in the pathogenesis of CD. Oxidative damage in liver is associated with hepatic lipid metabolism, and may be affecting the absorption and transport mechanisms of alpha-tocopherol in this organ. TBARS is an early marker of lipid peroxidation in plasma and tissues.

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Oxidative stress, Pulmonary Toxicity and Progressive Fibrosis Induced by Exposure to Single-Walled Carbon Nanotubes

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Nanotechnology is a fast growing field with emerging development of modern materials for electronics and information technology industries, cosmetics, health and medicine and in a variety of manufactured goods. Single-Walled Carbon Nanotubes (SWCNT) with unique physico-chemical, electronic and mechanical properties are novel materials of technological importance. Pulmonary exposure to SWCNT revealed toxicity within the range of doses relevant to exposure limits established by regulatory agencies for fine size carbon particles. In C57BL/6 mice exposed to SWCNT, pulmonary toxicity was associated with a dose-dependent augmentation of biomarkers of cell injury and oxidative stress quantified by cell counts, total protein, lactate dehydrogenase and g-glutamyltranspeptidase activities, reduced level of GSH, total antioxidant reserve along with the accumulation of lipid peroxidation products found in bronchoalveolar lavage (BAL) fluid and in the lung. Markers of pulmonary cytotoxicity corresponded to early development of acute inflammation, collagen accumulation, and progressive fibrosis. In mice maintained on vitamin E deficient diet, SWCNT significantly enhanced collagen deposition as compared to the vitamin E sufficient group. Overall, our data suggest that pharyngeal aspiration of SWCNT elicited a robust acute inflammatory response with early onset of progressive pulmonary fibrosis whose expression and severity was associated with the intensity of oxidative stress in the lung of the exposed C57BL/6 mice. Acknowledgements: supported by NIOSH OH008282, NORA 92700Y.

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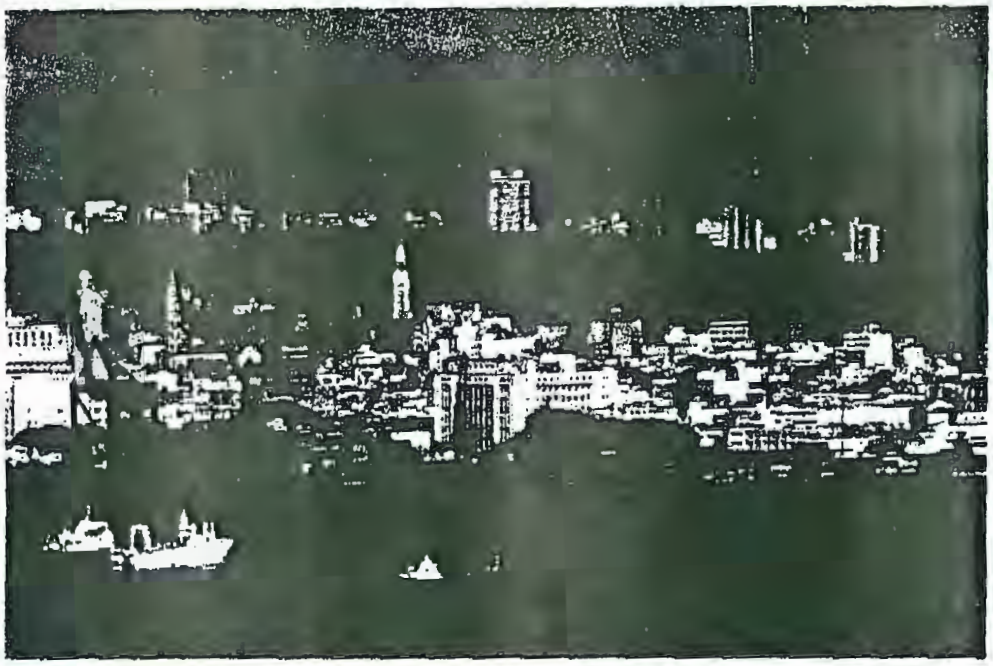

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