treated (n=5) or Ang II-infused (0.7 mg·kg^{-1}·day^{-1}; n=5) groups. DMPO was subcutaneously administered daily 96 hr before tissue analysis. Ang II infusion significantly increased systolic arterial blood pressure (196 ± 27 vs. 108 ± 24 mmHg; $p < 0.05$), as well as Na^+ excretion and urinary volume. Aortic tyrosine nitration and DMPO adduct formation were significantly increased in the endothelium of treated animals, pointing to the endothelium as the main target of oxidative stress. In renal tissue, nitrotyrosine and DMPO adducts were extensively increased at the luminal phase of tubular structures. Myeloperoxidase was also increased in renal tissues of treated animals, suggesting that a peroxidase-dependent mechanism of nitration was also occurring. Thus, DMPO immunospin trapping is a reliable and sensitive indicator of oxidative/nitrative tissue damage. Furthermore, ang II-induced vascular and renal injuries involves nitration mechanisms.

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CYTOCIDAL ACTIVITY OF LYSOPHOSPHATIDIC ACID ON CEREBRAL MICROVASCULAR ENDOTHELIAL CELLS IS POTENTIATED BY INTERLEUKIN-1 β

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Oxidant stress and inflammation play a significant role in hypoxic-ischemic injury to microvascular endothelial cells. Lysophosphatidic acid (LPA) is an inflammatory bioactive lipid peroxidation byproduct; its concentration increases during oxidant stress, upon activation of phospholipases. We examined the effects of LPA on neuromicrovascular cell survival and assessed if the latter is modulated by the pro-inflammatory cytokine Interleukin-1β (Il-1β). LPA induced a dose- and time-dependent death of porcine cerebral microvascular endothelial cells (PCEC). Human umbilical vein endothelial cells were also sensitive to LPA while smooth muscle and astroglial cells were minimally affected. LPA-induced PCEC death was prevented by pre-treatment with LPA_1 receptor antagonist THG1603 but seemed G_i-independent (not responsive to PTX). LPA-treated PCEC were swollen and did not exhibit features of apoptosis (nuclear condensation and TUNEL-positivity). Intracellular GSH levels decreased by 50% in presence of LPA and pre-treatment with n-acetyl-cysteine prevented LPA-induced cell death by 70-75%. Inhibition of COX-2, iNOS, $cPLA_2$ or lipoxygenase did not affect LPA's cytocidal activity. LPA activated JNK and p38 MAPK and these MAPKs contributed to the cell death (prevented by inhibitors, SP600125 and SB203580). Il-1β potentiated effects of LPA on calcium fluxes and PCEC death but did not alter LPA_1 receptor expression. More importantly, exposure of brain explants to LPA caused a significant reduction (35%) in microvessel density (lectin staining); this effect was potentiated by Il-1β. These novel data implicate LPA as a mediator of oxidative stress in neuromicrovascular endothelial cell death, which is potentiated by Il-1β.

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ROLE OF INDUCIBLE NITRIC OXIDE SYNTHASE-DERIVED NITRIC OXIDE IN LIPOPOLYSACCHARIDE PLUS INTERFERON-γ INDUCED PULMONARY INFLAMMATION

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Exposure of mice to lipopolysaccharide (LPS) plus interferon-γ(IFN-γ) increases nitric oxide (NO) production in the lung (Zeidler et al., 2003). The objective of the present study was to determine the role of inducible nitric oxide (iNOS)-induced NO in the pulmonary inflammatory response to LPS + IFN-γ. Male mice (iNOS knockout vs. C57BL/6J wild type; 8-10 weeks old) were exposed to LPS (1.2 mg/kg) + IFN-γ (5000 U/mouse) or to saline by pharyngeal aspiration, and pulmonary markers of damage and inflammation were compared at 24 and 72 hr post-exposure. The response at 24 hr post-exposure was marked by a decrease in total antioxidant capacity, increased alveolar macrophages, polymorphonuclear leukocytes, lactate dehydrogenase activity, albumin, tumor necrosis factor-α and macrophage inflammatory protein-2 in the acellular bronchoalveolar lavage fluid, and enhanced zymosan-stimulated chemiluminescence from alveolar macrophages to the same extent in both wild type and iNOS knockout mice. However, at 72 hr post-exposure the decline in antioxidant levels and the increase in markers of pulmonary damage and inflammation, and macrophage oxidant production were significantly greater in iNOS knockout vs. wild type mice. These data suggest that iNOS-derived NO plays an anti-inflammatory role in modifying the pulmonary response to LPS + IFN-γ.

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METALLOPORPHYRIN ANTIOXIDANT INHIBITS TH2 CELL IMMUNE RESPONSES

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Asthma is an airway inflammatory disease mediated by Th2 immune responses. Airway inflammation may exaggerate the production of oxidants suspected of being involved in the pathogenesis of asthma. The superoxide dismutase mimetic, AEOL10113, was demonstrated to inhibit OVA-induced airway inflammation in mice. We hypothesize that AEOL10113 may alter dendritic cell-dependent Th2 immune responses. Bone marrow stem cells were cultured with GM-CSF and IL-4 to generate $CD11c^+$ dendritic cells. OVA-specific Th2 cells were derived from $CD4^+$ $OVA_{323-339}$-specific T cell from DO11.10 mice. AEOL10113 was added to half of the dendritic cell and Th2 cell cultures for 3 days before co-culturing of the two cell types in the present of $OVA_{323-339}$. Th2 immune responses were determined by Th2 cell proliferation and cytokine production. We found that AEOL10113 pre-treatment of DC inhibited IL-4 and IL-5 productions by OVA-specific Th2 cells. Pre-treatment of Th2 cells with AEOL 10113 had no effect. Pre-treatment of either dendrtic cells or Th2 cells with the antioxidant had no effect on Th2 cell proliferation. However, addition of AEOL 10113 to the media during co-culturing inhibited Th2 cell proliferation. AEOL 10113 also inhibited Th2 cell proliferation when Th2 cells were cultured in plates coated with anti-CD3 and anti-CD28 without dentritic cells. These results suggest that the metalloporphyrin antioxidant, AEOL10113, attenuates antigen specific Th2 immune responses. The antioxidant modification of Th2 productions of IL-4 and IL-5 is dentritic cell dependant while its effect on Th2 cell proliferation does not appear to involve dentritic cells.

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MECHANISM OF SELECTIVE INHIBITION OF ENDOTHELIN-1 PRODUCTION IN BRAIN CAPILLARY ENDOTHELIAL CELLS BY N-ACETYLCYSTEINE

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N-acetylcysteine (NAC) is neuroprotective in animal models of bacterial meningitis, traumatic brain injury and cerebral ischemia, despite the fact that it poorly penetrates the blood-brain barrier. There is increasing evidence that reactive oxygen species (ROS) regulate gene expression of endothelin-1 (ET-1), a potent vasoconstrictor involved in the decline of cerebral blood flow in these disorders. We therefore studied whether NAC inhibits TNF--induced ET-1 production in rBCEC4 rat brain capillary

INFLAMMATION