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N-acetylcysteine selectively inhibits TNF-alpha–induced endothelin-1 upregulation in cerebrovascular endothelial cells

M.D. Sury[1], M. Frese-Schaper[2], M.K. Mühlemann[2], F.T.S. Schulthess[2], I. Blasig[3], M. Täuber[2], S. Shaw[4], S. Christen[5];
[1]University of Bern, Institute for Infectious Diseases, 3010 - Bern/CH, [2]University of Bern, Institute for Infectious Diseases, Bern/CH, [3]Leibniz-Association, Institute for Molecular Pharmacology, Berlin/DE, [4]University of Bern, Department of Clinical Research, Bern/CH, [5]Infectious Diseases, University of Berne, Bern/CH

Purpose: As a potential explantation for why N-acetylcysteine (NAC) is neuroprotective in acute brain injury models, despite the fact that it poorly penetrates the blood-brain barrier, we studied the effect of NAC on the upregulation of endothelin-1, an important factor of acute brain injury and whose upregulation is under the control of redox-sensitive signaling pathways.

Material and methods: EMSA, ELISA, quantitative RT-PCR, inhibitors, Western blot, kinase assay

Results: NAC dose-dependently inhibited TNF-α induced upregulation of ET-1 in rBCEC4 rat brain capillary endothelial cells. However, upregulation of inducible nitric oxide synthase (iNOS) was unaffected, despite the fact that is also dependent on NF-κB. In line with these results, activation of NF-κB (i.e., IκB degradation, nuclear p65 translocation and Ser536 phosphorylation) was unaffected by NAC. However, inhibition of NF-κB DNA-binding activity in the early phase of stimulation suggested that NAC may inhibit ET-1 upregulation by inhibiting a pathway that modulates NF-κB activity. Indeed, NAC inhibited phosphorylation of p38, kinase activity of its downstream target MSK-1, and p65 Ser276 phosphorylation, while ET-1 upregulation was selectively inhibited by the p38 inhibitor SB203580 and MSK-1 inhibitor H-89.

Conclusion: The results suggest that NAC selectively inhibits ET-1 upregulation by inhibiting p38–MSK-1-dependent transactivation of NF-κB.

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Carbon Nanotube Exposure Caused Induction of Oxidative Stress, Pulmonary Injury And Fibrosis

A. Shvedova[1], E. Kisin[2], A. Murray[3], D. Schwegler-Berry[4], V. Castranova[5], V.E. Kagan[6], Y. Tyurina[6];
[1]/, [2]Pathology and Physiology Research Branch, NIOSH/CDC, Morgantown/US, [3]Physiology and Pharmacology Departement, WVU, Morgantown/US, [4]Pathology and Physiology Rsearch Departement, NIOSH/CDC, Morgantown/US, [5]Pathology and Physiology Branch, NIOSH/CDC, Morgantown/US, [6]Center for Free Radical & Antioxidant Health and Departement of Enviremental Health, University of Pittsburgh, Pittsburgh/US

Single-walled carbon nanotubes (SWCNT) are new members of carbon allotropes with potentially broad range of revolutionary applications. Unprocessed SWCNT could become airborne and potentially reach lungs. Yet their pulmonary toxicity remains poorly characterized. Pharyngeal aspiration of SWCNT elicited unusual pulmonary effects in C57BL/6 mice that combined a robust but acute inflammation with early onset yet progressive fibrosis. A dose-dependent increase in the protein, lactate dehydrogenase (LDH), and g-glutamyl transferase (GGT) activities in BAL were found along with accumulation of 4-hydroxynonenal (oxidative biomarker), and depletion of glutathione in lungs. An early neutrophils accumulation (day 1), followed by lymphocyte (day 3) and macrophage (day 7) influx were accompanied by early elevation of pro-inflammatory cytokines (TNF-a, IL-1b, day 1) followed by fibrogenic TGF-b1 (peaked on day 7). A rapid progressive fibrosis found in mice exhibited two distinct morphologies: (1) SWCNT-induced granulomas mainly associated with hypertrophied epithelial cells surrounding SWCNT aggregates and (2) diffuse interstitial fibrosis and alveolar wall thickening likely associated with dispersed SWCNT. In vitro exposure of murine RAW264.7 macrophages to SWCNT triggered TGF-b1 production similarly to zymosan but generated less TNF-a and IL-1b. SWCNT did not cause superoxide or NO• production, active SWCNT engulfment, or apoptosis in RAW264.7 macrophages.

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The effect of nitric oxide on the apoptosis of Jurkat T cells

J. Tarr[1], P. Eggleton[2], R. Haigh[3], P. Winyard[4];
[1]IBCS, Peninsula Medical School, Ex1 2LU - Exeter/GB, [2]Institute of biomedical and clinical science, Peninsula Medical School, EX1 2LU - Exeter/GB, [3]Consultant rheumatologist, Princess Elisabeth Orthapeadic Centre, Royal Devon and Exeter Health Care Trust (Wonford), EX2 5DW - Exeter/GB, [4]St. Luke's Campus, Peninsula Medical School, Exeter/GB

Purpose: Rheumatoid arthritis (RA) is an inflammatory disease affecting synovial joints, which is characterised by T cell accumulation. Resolution of 'normal' inflammation involves the removal of T cells by apoptosis. In RA, autoreactive T cells appear to be apoptosis resistant. Nitric oxide ($NO^{\cdot}$) has been implicated in this resistance, and we are investigating the effect of NO on apoptosis of T cells.

Material and methods: Jurkat T cells treated with TNF-α were exposed to different concentrations of NO donor. Release of NO from NO donors was measured using flow injection analysis (FIA) and electron paramagnetic resonance (EPR) spectrometry. Apoptosis was assessed using an annexin-V detection kit.

Results: Application of NO at low concentrations to TNF-α treated cells had an anti-apoptotic effect, whereas high concentrations of NO caused increased levels of apoptosis.

Conclusion: NO has a biphasic effect on the apoptosis of Jurkat T cells. This phenomenon may contribute to the complex pro- and anti-inflammatory effects of nitric oxide synthase (NOS) inhibitors in arthritis models.

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8-Hydroxydeoxyguanosine Suppresses LPS-induced NO Production and COX-2 Activity Through Inhibition of Rac1/STATs Signal Cascade in Brain Microglia

H.S. Kim, J.E. Kim, M. Chung, S.K. Ye;
Department of Pharmacology, Seoul National University College of Medicine, 110 - 799/KR

Purpose: Our previous reports showed that oh^{8}dG present in cytosol have antioxidant property. However, it is unknown about cytosolic action and function of oh^{8}dG, a nucleoside of oh^{8}G.

Material and methods: The effects of oh^{8}dG are analyzed though immunobloting, RT-PCR in activated BV2 microglia cells. The inhibition of transcriptional regulation of iNOS and COX-2 gene by oh^{8}dG are studied in LPS-stimulated CHO cell though luciferase assay and ChIP assay. To confirm effect of oh^{8}dG, systemic inflammatory-induced mouse models are used.

Results: We investigated the effect of oh^{8}dG on LPS-elicited microglial stimulation and signaling cascades. The results showed that oh^{8}dG suppressed LPS-induced iNOS and COX-2 protein expression, and NO production and COX-2 activity. Also, oh^{8}dG inhibited JAK/STATs signaling through suppression of NADPH oxidase activity, Rac1 activity. In addition, luciferase reporter gene assays showed oh^{8}dG suppressed iNOS and COX-2 transcriptional expression via Rac1/STATs signal cascade. We also found that oh^{8}dG blocked recruitment of STAT1, STAT3 and p300 and acetylation of H3 in iNOS and COX-2 promoter.

Conclusion: Taken together, these results suggest that oh^{8}dG suppressed NO production and COX-2 activity via inhibition of Rac1/STATs signal cascade. This study provides new insights into the potential beneficial role of oh^{8}dG in brain microglial responses to LPS during inflammation.

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