411.7

SILICA-INDUCED NUCLEAR FACTOR-κB ACTIVATION: INVOLVEMENT OF REACTIVE OXYGEN SPECIES AND PROTEIN TYROSINE KINASE ACTIVATION.

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Nuclear Factor-κB(NF-κB) is a multiprotein complex that may regulate a variety of inflammatory cytokines involved in the initiation and progression of silicosis. The present study documents the ability of in vitro silica exposure to induce DNA-binding activity of NF-κB in mouse macrophages(RAW264.7 cells) and investigates the role of reactive oxygen species(ROS) and/or protein tyrosine kinase in this activation. In vitro exposure of mouse macrophages to silica(100 μg/ml) resulted in a 2-fold increase in ROS production, measured as the generation of chemiluminescence(CL), and caused activation of NF-κB. Silica-induced CL was inhibited 100% by superoxide dismutase(SOD) and 75% by catalase, while NF-κB activation was inhibited by a variety of antioxidants(catalase, SOD, sodium formate, α-tocopherol, and pyrrolidine dithiocarbamate). Further evidence of the involvement of ROS in NF-κB activation is that 1 mM H_2O_2 enhanced NF-κB/DNA binding and that this activation was inhibited by catalase. Specific inhibitors of protein tyrosine kinase, such as herbimycin A, genistein and AG-494, prevented NF-κB activation in silica-treated cells. Genistein and AG-494 also prevented NF-κB activation in H_2O_2-treated cells. In contrast, inhibitors of protein kinase A or C, such as H89, staurosporin, calphostin C, chelerythrine, and H7 had no inhibitory effect on this response. The results suggest that ROS play a role in silica-induced NF-κB activation in macrophages and that phosphorylation events mediated by tyrosine kinase may be involved in this activation.

411.8

ENHANCED PRODUCTION OF PROSTAGLANDIN E_2 (PGE_2) BY ALVEOLAR MACROPHAGE (AM) HARVESTED FROM SILICA-EXPOSED RATS: POSSIBLE REGULATORY ROLE OF NITRIC OXIDE (NO).

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This study investigated whether NO plays a significant role in regulating the production of PGE_2 or inflammatory cytokines by silica-exposed AM. Rats were treated by intratracheal instillation of saline (control) or silica (20 mg/ rat) and AM harvested by bronchoalveolar lavage at 1, 3, 7, 14, and 28 days post-exposure. AM were cultured for 24 hr and ex vivo production of PGE_2, leukotriene B_4 (LTB_4), TNF-α, IL-1, and NO were monitored. In vivo exposure to silica resulted in an increase in PGE_2 production which peaked (185 fold) at 3 days post-exposure, while LTB_4 release was decreased. Silica exposure increased NO production by 9 fold at 3 days post-exposure, while causing a moderate increase in TNF-α production and a significant elevation of IL-1 secretion which was sustained through 28 days post-exposure. Tetrandrine, an antifibrotic agent, inhibited IL-1 and TNF-α production but did not affect NO or PGE_2 release. Indomethacin blocked PGE_2 production completely and had a moderate effect on NO production. Furthermore, inhibition of NO production with N-nitro-L-arginine methyl ester enhanced PGE_2 production without affecting TNF-α or IL-1 release. These results suggest that silica-induced stimulation of PGE_2 production is controlled via a different pathway than cytokine production and that NO may play a role in limiting PGE_2 production in silica-exposed rats.

411.9

Effects of Peroxynitrite on the Pulmonary Oxidative State and Vascular Permeability.

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Excess nitric oxide (NO) formation especially in the presence of superoxide anion (O_2^-) leads to the formation of peroxynitrite ($OONO^-$) which may result in lung injury. Oxidant-mediated lung injury has a critical role in pulmonary diseases. We therefore determined whether $OONO^-$ affects pulmonary vascular permeability, lipid peroxidation and formation of nitrotyrosine using an isolated perfused rat lung model. The lung weight gain during bolus $OONO^-$ infusion increased in a dose-dependent manner over a range of 3-30 μmoles. $OONO^-$ increased the production of thiobarbituric acid reactive substances, an index of lipid peroxidation. Furthermore, nitrotyrosine levels in lung tissue rose with increased concentration of $OONO^-$ as determined by western blot using anti-nitrotyrosine antibodies. These results suggest that $OONO^-$, formed from NO and O_2^-, leads to increased pulmonary vascular permeability possibly through lipid peroxidation and/or nitration of cell membrane proteins. (Supported by the UNC Institute of Nutrition and NIH 52146).

ISCHEMIA/REPERFUSION INJURY (412.1-412.2)

412.1

TNFα REQUIRES KUPFFER CELLS TO INITIATE HEPATIC INJURY FOLLOWING HINDLIMB ISCHEMIA IN THE RAT.

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We tested the hypothesis that TNFα indirectly initiates hepatic injury through the activation of Kupffer cells following a remote ischemic insult. We applied intravital fluorescence microscopy to the livers of normotensive rats following 4-hrs of hindlimb ischemia/reperfusion (I/R). After 3-hrs of reperfusion, hepatic injury was determined based on measures of lethal hepatocyte injury (#/10^{-2} mm^2) using propidium iodide (0.05 mg/100g BW), and the number of continuously perfused sinusoids (% of total sinusoids). Rats were randomly allocated to test the role of Kupffer cells (n=4), TNFα (n=4), or the interaction between TNFα and Kupffer cells (n=4) by: i) a pretreatment with gadolinium chloride 24-hrs prior to ischemia ($GdCl_3$; 1 mg/100gBW); ii) a polyclonal antibody against TNFα administered prior to reperfusion ($3.5x10^4$ neutralizing units/100gBW); iii) or a combination of the treatments. Sham rats (n=5) received limb ischemia only. Systemic TNFα, measured by ELISA, peaked at 30 minutes of reperfusion (60.5±22.2 pg/ml), returning to baseline within 90 minutes. In all treatment groups, injury was reduced at least 2-fold (Sham: 158.5±21.1 vs. TNFα PAb: 61.8±32.4 vs. $GdCl_3$: 59.7±21.7; $p<0.05$). The reduction of Kupffer cell function, or the inhibition of TNFα resulted in a 15-20% increase in the number of continuously perfused sinusoids, with no appreciable difference observed between treatments. No additional benefits were provided by the combination of treatments. In additon, TNFα, in the absence of Kupffer cell function, did not appear to initiate significant hepatic parenchymal or microvascular injury. These results suggest that TNFα requires Kupffer cells to initiate remote hepatic injury following limb ischemia. *Supported by MRC Canada & LHSC.*

412.2

PRESENILATION SIGNS IN RATS AFTER 10-MINUTES TOTAL ISCHEMIA.

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Complex of pathologic and compensatory reactions after 10-minutes total ischemia leads to development of organism presenilation.

Investigations on the white rats one year after total ischemia revealed decrease of motional and orientative-investigative activity, that of ability for memorizing of trined reflexes and their preservation, decrease of muscular strength. Besides cholesterol accumulation in the important living organs and tissues, development of atherogenesis dislipidemia, oxidative modification of lipoproteins were observed.

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ABSTRACTS
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