nitrate, from endogenous or dietary sources, have emerged as alternative substrates for NO formation in mammals. Here we investigated the in vivo effects of inorganic nitrate and nitrite on microvascular inflammation.
Anesthetized mice, pre-treated with sodium nitrate (0.85 g/L in drinking water, 7 days) or sodium nitrite (1.3 mg/kg, iv), were investigated for leukocyte recruitment (rolling, adhesion and emigration) in postcapillary venules of the cremaster muscle by intravital microscopy. The effects of dietary nitrate on the ability to clear a *S. aureus* infection were studied in mice by non-invasive IVIS imaging.
Leukocyte emigration in response to the pro-inflammatory chemokine MIP-2 was reduced by 70 % after dietary nitrate treatment as well as by acute nitrite administration. Acute nitrite administration also reduced leukocyte adhesion to a similar extent and this effect was normalized by the sGC inhibitor ODQ, while the effect on emigrated leukocytes was not altered by this treatment. Despite attenuation of the acute immune response, the over-all ability to clear an infection was not suppressed by dietary nitrate.
We conclude that inorganic nitrate and nitrite reduces leukocyte recruitment in a mouse model of microvascular inflammation. This effects was partly cGMP-dependent and likely involves formation of NO and other bioactive nitrogen oxides.

doi: 10.1016/j.freeradbiomed.2010.10.400

391

Iron Overload induces Oxidative Stress in Macrophages Hampering Their Physiology

***Gurpreet Kaur*[1]**
[1] *F/o Science, Jamia Hamdard, New Delhi, India*

The present study was designed to corroborate the hypothesis that iron overload associated oxidative stress weakens the physiology of phagocytes jeopardizing the immunity of body enhancing the risk of infections. Rats were iron overloaded by administering iron dextran (7.5 mg/day, i.m.) for 6 weeks. Their peritoneal macrophages were isolated and assessed for iron accretion, redox modulation and physiological alterations. Macrophages accumulated a marked amount of iron paralleled by ROS upsurge that overwhelmed their antioxidant armory. Antioxidant defense of cells significantly deminished and oxidative damage was induced. Concomitant to that, modulation was observed in secretion of proinflammatory mediators and proinflammatory enzymes in LPS stimulated macrophages from iron overloaded animals. Investigation of redox sensitive upstream transcription factor, NFκB, revealed iron overload to have augmented its activation. A significant impairment was also observed in phagocytic function of macrophages. Mannose receptor (MR), a major phagocytic receptor, exhibited hampered recycling with a marked attenuation in number at surface. Ligands internalized via MR also showed a diminished degradation following iron overload. The results of the present study imply that oxidative stress induced macrophage dysfunctioning may modulate the immune response of iron overload patients.

doi: 10.1016/j.freeradbiomed.2010.10.401

392

Iron-Mediated Free Radical Formation by Aged PRBC Units: Implications for Clinical Transfusion

John Cavaretta[1,2], Chenell Donadee[1,2], Mark T. Gladwin[1,2], Janet S. Lee[1,2], and ***Eric E. Kelley*[2,3]**
[1 2 3] *University of Pittsburgh*

Recent reports demonstrate an increased incidence of adverse clinical outcomes including renal, respiratory, and/or multiple organ failure associated with transfusion of aged packed red blood cell (PRBC) units. The biochemical and morphologic changes that RBCs undergo during standard blood bank conditions result in time-dependent loss of membrane integrity/hemolysis and are implicated in promoting vascular oxidative stress as well as exacerbating pre-existing inflammation. However, the kinetics and source of free radical formation in PRBC units are not defined. Therefore, we examined temporally the free radical generating capacity of PRBC (adsol-preserved, non-leukoreduced) using EPR spin trapping. A time-dependent increase in $CP^{\bullet}$ formation was observed when the supernatant from PRBC units (1-6 wks of storage) was exposed to the spin probe CPH and analyzed in the EPR cavity at 37ºC for 10 min. Neither addition of the antioxidants SOD and CAT nor the presence of the inhibitors (DPI, L-NAME, Uloric and allopurinol) significantly altered the $CP^{\bullet}$ signal. However, addition of deferoxamine (DFO) reduced $CP^{\bullet}$ formation in a concentration-dependent manner (1-100 μM) indicating free Fe as the seminal reactant. In addition, reduction of ferric iron (Fe^{3+}) to the more reactive ferrous iron (Fe^{2+}) iron by incubation with L-ascorbic acid significantly enhanced the $CP^{\bullet}$ signal ($Fe^{3+} + AscH^{-} \rightarrow Fe^{2+} + Asc^{\bullet-}$) further verifying the presence of free iron. This process also resulted in a storage time-dependent enhancement of ascorbyl radical ($Asc^{\bullet-}$) formation that was abrogated by DFO. These data demonstrate that free Fe may be contributory to storage lesion-mediated toxicity and, as such, treatment approaches to chelate this undesirable Fe may limit iatrogenic complications currently encountered when transfusing aged PRBC.

doi: 10.1016/j.freeradbiomed.2010.10.402

393

Oxidative Stress and Trichloroethene-Mediated Autoimmunity

Gangduo Wang[1], Huaxian Ma[1], Xiuzhen Fan[1], Jianling Wang[1], G.A.S. Ansari[1], and ***M. Firoze Khan*[1]**
[1] *University of Texas Medical Branch*

Exposure to trichloroethene (TCE), a ubiquitous environmental contaminant, is known to induce an autoimmune response both in humans and animal models. However, mechanisms underlying TCE-mediated autoimmunity remain largely unknown. Previous studies from our laboratory in $MRL^{+/+}$ mice suggest that oxidative stress may contribute to TCE-induced autoimmunity. The current study was aimed to further assess the role of oxidative stress in TCE-induced autoimmunity. Groups of female $MRL^{+/+}$ mice were given TCE, N-acetylcysteine (NAC) or TCE along with NAC for 6 weeks (TCE, 10 mmol/kg, i.p., every 4th day; NAC, 250 mg/kg/day through drinking water). Significant increases in the serum levels of anti-nuclear-, anti-Sm- and anti-dsDNA-antibodies were observed following TCE exposure. TCE exposure also led to significant induction of anti-hydroxynonenal (HNE)- and anti-malondialdehyde (MDA)-protein adduct antibodies in the sera along with increased protein oxidation (carbonylation) and decreased glutathione (GSH) levels in the sera, livers and kidneys, suggesting an overall increase in oxidative stress. Remarkably, NAC supplementation not only attenuated the TCE-induced oxidative stress, but also the markers of autoimmune response, as evident from their decreased levels in the sera. Our results further support a role of oxidative stress in TCE-induced autoimmune response. Attenuation of TCE-induced autoimmune response in mice by NAC could be important in developing preventive strategies.
Supported by NIH ES016302.

doi: 10.1016/j.freeradbiomed.2010.10.403