PROTECTIVE EFFECT OF WR-1065 AGAINST H_2O_2 INJURY. Slosman DO, Jolliet Ph, Donath A, Polla BS. Hôpital Cantonal Universitaire de Genève. 1211 Geneva 4, SWITZERLAND.

Generation of reactive oxygen species (ROS) has been suggested to mediate toxic and inflammatory lung injury. Because a radioprotective agent, the organic phosphothioate WR-1065, has been shown to protect in vitro from cellular oxidative injury (Polla et al; BiochemPharmacol 40:1469-,1990), we hypothesized that WR-1065 could protect against ROS during the early phase of lung injury when endothelial cell lesions appear. To assess endothelial cell function, lung extraction (%E) of a norepinephrine analog, the labeled (I-123) metaiodo-benzylguanidine (MIBG) (Slosman et al; EJNuclMed 16:633-,1990), was measured in an isolated-perfused rat lung model. Labeled (I-125) human serum albumin (HSA) was perfused simultaneously to MIBG to test for lung edema. In control experiments, after 10 minutes of perfusion with krebs-ringer bicarbonate medium (Mn), lungs were perfused for 2 minutes with Mn+MIBG+HSA and exhibited a normal %E-MIBG of 21.7±3.8% (n=7). When increasing concentrations of H_2O_2 (0.025,0.125,0.5 and 2 mM) were added to Mn for the overall procedure, we observed a progressive reduction of E%-MIBG, which was significantly different from normal at 2 mM (10.2±5.0%,n=7) (ANOVA,$p<0.005$,n=28). To test the protective effect of WR-1065 and to avoid direct interaction between WR-1065 and H_2O_2, the perfusion procedure was divided in 3 phases. The perfusate in phase 1 was Mn ± WR-1065 (2mM); in phase 2, the perfusate was Mn+H_2O_2 (2mM) and in phase 3, the perfusate was Mn+MIBG+HSA whereas the time of perfusion of the 3 phases were respectively 8, 2 and 2 minutes. E%-MIBG extraction was 8.7±7.4% after the 2 minutes of perfusion of H_2O_2 when Mn was free of WR-1065 in phase 1. The addition of WR-1065 in phase 1 normalized E%-MIBG (18.2±5.0%,n=6) which was significantly increased (ANOVA,$p<0.005$, n=21) as compared to H_2O_2 alone and not different from normal conditions. E%-HSA was never modified and remained below 2% in all experiments. Our results demonstrate that WR-1065 is able to protect against ROS lung injury in an isolated-perfused rat lung model as assessed by endothelial cell function monitoring. Therefore, this radioprotector agent could be potentially useful to prevent lung injury mediated by ROS such as bleomycin lung toxicity.

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BIOCHEMICAL CHANGES IN LUNG LAVAGE FLUID AND ALTERATION IN MACROPHAGE FUNCTION IN RATS EXPOSED TO HYPEROXIA. HV Dedhia, V Castranova, JYC Ma, NS Dalal, D Banks, JKH Ma, EB Flink, V Vallyathan, M Billie. Division of Pulmonary & CCM, Department of Medicine, Anesthesiology and Chemistry, W. V. University. and Division of Respiratory Disease Studies, NIOSH, Morgantown, WV 26505, USA.

The aim of this study was to characterize selected pulmonary changes by analyzing lung lavage fluid and pulmonary macrophage (AM) functions in rats exposed to hyperoxia (FiO_2 >.95) for 64 hrs (a model of ARDS). A total of 18 Sprague Dawley rats were exposed and 14 served as controls. After sacrifice, bronchoalveolar lavage fluid was analyzed for total and differential cell counts (N = 32), protein and phospholipid content (N= 13), cellular viability (N = 7), lipid peroxidation (LP, N = 8), and glutathione levels (N = 8). AM function (N = 13) was assessed by chemiluminescence (CL), hydrogen peroxide (H_2O_2) production (N = 9), and hydroxyl radical (OH) formation (N = 7) measured by electron spin resonance. Cell spreading (N = 10) was measured by scanning electron microscopy. Hyperoxia did not affect AM spreading, phospholipid content of lavage fluid, or glutathione content of lavaged cells. However, statistically significant changes ($p < 0.05$), were seen in hyperoxic vs air control rats: elevated protein content (1.67 vs 0.29 mg/ml); increased PMN (1.43 vs 0.76 x 10^6 cell/rat), lymphocyte (1.86 vs 0.33 x 10^6 cell/rat), and RBC (3.75 vs 0.93 x 10^6 cell/rat); reduction in AM (4.84 vs 12.7 x 10^6 cell/rat) counts; and increased LP (2.2 times control). The total cell count and AM viability were reduced from 15.7 to 12.2 x 10^6 cell/rat and 90 to 80 % respectively in the hyperoxic rats but were not statistically significant. Zymosan stimulated H_2O_2 (N = 9) release and OH radical production were decreased 41 % and 53 %, respectively, after hyperoxia. In contrast, zymosan stimulated CL was elevated by 540 % in hyperoxic AM. The CL generated by AM from oxygen exposed and control animals showed no significant difference in inhibition by superoxide dismutase (↓O_2^-), indomethacin (↓cyclooxygenase), catalase (↓H_2O_2) or A 63162 (↓lipoxygenase). We conclude that hyperoxic treatment of rats for 64 hr results in increased lipid peroxidation, alveolar hemorrhage, abnormal capillary permeability and decreased membrane integrity of pulmonary cells. The ability of phagocytes to produce H_2O_2 and hydroxyl radicals was impaired after 64 hr of hyperoxia. The data indicate that high CL in hyperoxic alveolar macrophages may be due to reactive lipid peroxidation products generated by oxygen exposure.

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SUSCEPTIBILITY TO 0.3 PPM OZONE-INDUCED INFLAMMATION IS GENETICALLY DETERMINED IN INBRED MICE. S.R. Kleeberger, R.C. Levitt, and L.-Y. Zhang. School of Hygiene and Public Health, The Johns Hopkins University. Baltimore, MD 21205.

We demonstrated previously that C57BL/6J (B6) inbred mice are susceptible, and C3H/HeJ (C3) mice are resistant to the airway inflammatory response induced by an acute (3 hr) exposure to 2 ppm ozone (O_3). The response was determined to be controlled by a single gene at the Inf locus. In the present study we characterized the airway inflammatory response to sub-acute exposures to environmentally relevant concentrations of O_3 in B6 and C3 mice, and investigated potential genetic susceptibility to these exposures. Male B6 and C3 mice (20-25 g, 5-7 wk) were exposed continuously to 0.12 ppm O_3, 0.3 ppm O_3, or filtered air (control). Pulmonary inflammation was assessed after 24, 48, and 72 hr by differential cell count and total protein (a marker of epithelial permeability) in bronchoalveolar lavage (BAL) returns. After 48 hr exposure to 0.3 ppm O_3, the maximum influx of polymorphonuclear leukoctyes (PMNs) was significantly greater ($P<0.001$) in B6 mice (10.1±0.5 x 10^3/ml) as compared to C3 (2.4±0.3 x 10^3/ml). Maximal mean total BAL protein was significantly different after 72 hr exposure (483±70 μg/ml [B6] vs 228±26 μg/ml [C3]; $P<0.001$). Minimal inflammation developed after 72 hr exposure to 0.12 ppm O_3 in C3 and B6 mice, and no differential susceptibility between strains was detected. To further evaluate the potential genetic contribution to the inflammatory response to 0.3 ppm O_3, the F_1 and F_2 progeny from crosses between C3 and B6 progenitors were examined. The O_3-induced changes in PMNs of F_1 mice (N=20) were not different from the C3 progenitor and were phenotyped as resistant. The phenotypes of these progeny were consistent with the hypothesis that a single autosomal recessive gene confers susceptibility to 0.3 ppm O_3-induced inflammation. Continued genetic characterization of this model of O_3-induced airway inflammation will provide further insight into mechanisms of susceptibility.
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AIRWAY INFLAMMATORY RESPONSES TO 2 PPM O_3 AND 0.3 PPM O_3 ARE CONTROLLED BY DIFFERENT LOCI. S.R. Kleeberger, R.C. Levitt, and L.-Y. Zhang. School of Hygiene and Public Health, The Johns Hopkins University. Baltimore, MD 21205.

Acute exposure to 2 ppm ozone (O_3) induces differential airway inflammation in susceptible C57BL/6J (B6) and resistant C3H/HeJ (C3) inbred strains of mice. This response is controlled by a single autosomal gene at the Inf locus. We also demonstrated that inflammatory responses elicited by sub-acute 48 hr exposure to 0.3 ppm O_3 may be controlled by a single gene in B6 and C3 mice (ATS, 1992). In the present study, we began to determine whether the inflammatory responses to 2 ppm O_3/3 hr and 0.3 ppm O_3/48 hr are similarly controlled by Inf. To test this hypothesis, BXH Recombinant Inbred (RI) mice were phenotyped for their inflammatory responses to 0.3 ppm O_3, and the phenotypes were compared by cosegregation analysis to previously determined phenotypes for responses to 2 ppm O_3. Age- and sex-matched BXH RIs were exposed continuously to 0.3 ppm O_3 or filtered air (control), and pulmonary inflammation was assessed after 48 hr by counting polymorphonuclear leukocytes (PMNs) in bronchoalveolar lavage returns. Phenotypes were assigned to each BXH RI by comparing statistically their inflammatory responses to those of progenitors. A 4.2-fold difference in lavageable PMNs was found between resistant C3 and susceptible B6 mice after 48 hr exposure to 0.3 O_3 ($P<0.001$). The mean number of lavageable PMNs in 9 of 12 BXH RIs did not differ significantly (99% confidence) from progenitor C3 mice and were phenotyped as resistant. The inflammatory responses of 3 BXH RIs did not differ from B6 mice and were typed as susceptible. Comparison of this Strain Distribution Pattern (SDP; level of responsiveness of each BXH RI strain) with the previously determined SDP for Inf indicated that 9 RIs responded concordantly to the two exposures, and 3 RIs responded discordantly. Discordance of the RI SDPs suggests that different genes control the inflammatory responses to 2 ppm O_3 and 0.3 ppm O_3. Further characterization of the genetic control of O_3-induced airway inflammation will provide insight into the mechanisms which determine susceptibility to oxidant lung injury. Support: EPA R-816557, CIAR 90-27, and ES 03819.

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ABSTRACTS

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