Biochimica et Biophysica Acta 890 (1987) 271–274
Elsevier

BBA 40077

BBA Report

Age-related difference in pulmonary response to ozone

Mark R. Montgomery [a,b], Patricia Raska-Emery [a] and John U. Balis [a,c]

[a] *Medical Research Service, Veterans Hospital,* [b] *Department of Environmental and Occupational Health, College of Public Health, and* [c] *Department of Pathology, College of Medicine, University of South Florida, Tampa, FL (U.S.A.)*

(Received 31 July 1986)

Key words: Aging; Ozone; Lung; NADPH; $NADP^+$

Acute exposure to 1.5 ppm O_3 produced different responses in adult and aged rat lungs. Total triphosphonucleotides were only slightly decreased in adult animals, but were markedly decreased in aged animals. Also, adult animals maintained a greater proportion of their available triphosphonucleotides in the reduced form (NADPH) compared to aged animals. These results suggest that aged animals may not be able to maintain pulmonary reducing equivalents as efficiently as adult animals in the face of an oxidant insult.

In aging research, the lung has received very little attention to date, a point best made in several recent review articles that do not mention the lung [1–3]. In other reviews specifically concerned with aging and the lung, only a few sentences [4] or a few paragraphs [5] have addressed the question of biochemistry, with the remainder of the reviews being concerned with the structure-function relationships of elastin, collagen, and surfactant. Scattered reports appear throughout the literature on age-related alterations in various lung enzymes [6,7]. These reports only document changes in activities without attempting to define any positive or negative consequences. Clinical interest in drug metabolism has resulted in a voluminous literature on age-related changes in the hepatic system; however, no information is available on enzyme concentrations, specific activities, or availability of reducing equivalents for the drug metabolizing enzyme complex of the aged lung.

Numerous pulmonary diseases and conditions have age-related components; emphysema and cancer being the most common [8,9]. However, age alone does not necessarily precipitate these conditions. Certain chemical stresses accelerate cellular degeneration and death in the aged lung [10]. Many of these insults, such as oxygen toxicity, cigarette smoking, certain drugs, ionizing radiation, and several environmental pollutants (e.g., ozone) are classified as 'oxidants' or have distinct oxidation-reduction influences in their overall pulmonary toxicity. There appears to be no information on the effects of aging on reduction potential in the lung. Specifically, no systematic study is available concerning oxidant stress and pulmonary bioenergetics in the aged.

This report presents lung triphosphonucleotide concentrations and balance in adult and aged animals following acute exposure to ozone. The balance of [NADPH]/[$NADP^+$] may play a central role in cellular viability. Not only does NADPH provide reducing equivalents for maintenance of cell membranes (via reduced glutathione), but also it provides energy for numerous subcellular enzymes involved in catabolism of exogenous chemicals. The concentration of ozone selected for initial study, 1.5 ppm, represents a moderately severe exposure for an 8 h period (National Ambient Air Quality Standard is 0.1 ppm for 8 h exposure). This concentration was selected to pro-

Correspondence: M.R. Montgomery, Medical Research Service, Veterans Hospital, University of South Florida, Tampa, FL 33612, U.S.A.

vide a severe oxidant stress to the adult and aged animals; this exposure duration was selected to produce pulmonary injury with minimal complications from repair processes (Balis, J.U. and Montgomery, M.R., unpublished data).

Fischer 344, pathogen-free male rats (4–6 months old, 'adult') were obtained from Charles River Breeders and Fischer 344, pathogen-free retired male breeders (24–26 months old, 'aged') were obtained from Harlan Sprague–Dawley through an agreement with the National Institute on Aging. For determination of NADPH oxidation rates, lung microsomal fractions were prepared by standard ultracentrifugation techniques. The oxidation rate was determined as described by Jeffrey and Mannering [11] at A_{340} ($E = 6.22$ $mM^{-1} \cdot cm^{-1}$) with the inclusion of 1 mM 5'-AMP to inhibit pyrophosphatase activity. The rate of oxidation was stimulated by the addition of 2 mM benzphetamine hydrochloride.

Animals were exposed to 1.5 ppm of ozone for 8 h in an inhalation chamber [12] and were killed immediately upon removal from the chamber. Ozone was generated by passing 100% O_2 through an OREC Model 03V1-0 Ozone Generator (Ozone Research Equipment Co., Phoenix, AZ) and was mixed with compressed air in a ratio appropriate to produce 1.46 ± 0.06 ppm O_3 (mean ± S.E.M. for hourly averaged concentrations for 8 h). The total gas flow was 120 l/min and produced approximately one air exchange per min. The O_3 concentration within the chamber was monitored continuously on a Mast Model 727-3 Ozone Monitor (Mast Development Co., Davenport, IA) utilizing ultraviolet absorption at 254 nm in a 36 cm sample cell.

Lungs were perfused in situ with saline and one piece from each lobe (approx. 250 mg) was taken for NADPH and $NADP^+$ determination by the method of Slater and Sawyer [13], with modifications. Triphosphonucleotides were extracted as described by Witschi et al. [14], were determined colorimetrically by the method of Nisselbaum and Green [15], and were quantitated by comparison to a standard curve.

The capacity of pulmonary NADPH-dependent metabolism to respond to an oxidative metabolic stimulus was estimated by addition of a substrate (benzphetamine) for the microsomal mixed function oxidase enzyme complex. While there was no difference in the basal rates (no exogenously added substrate) between adult and aged animals (3.4 and 3.5 nmol NADPH oxidized per mg per min, respectively), addition of the substrate resulted in distinctly different responses. The rate of NADPH oxidation was increased 74% (±21%, S.E.M.) in adult rats and 159% (±28%, S.E.M.) in aged rats.

Extrapolation of any conclusions drawn from in vitro reaction rates to an in vivo situation is difficult. However, these results did suggest that there may be an age-related difference in the response of lung triphosphonucleotides to oxidative metabolic stress. Therefore, lung concentrations of NADPH and $NADP^+$ were determined in adult and aged rats following an 8 h exposure to 1.5 ppm O_3. The results are presented in Table I.

Adult and aged controls were not different in concentrations of NADPH or $NADP^+$ as expressed per g wet weight lung tissue. Aging did not influence the wet weight/dry weight ratio of the lungs (adult: 5.68 ± 0.34; aged; 6.10 ± 0.50), nor were these ratios altered significantly by the ozone exposure. Simultaneous measurement of lung DNA content also indicated no differences with aging or with exposure (data not shown).

The sum of lung triphosphonucleotides, [NADPH] + [$NADP^+$], and the ratio of reduced-to-oxidized, [NADPH]/[$NADP^+$], were not different for controls. These important base-line values show clearly that there is no age-related decrement in the basal concentrations or reduction state of this pyridine nucleotide. However, adult and aged rats responded differently to the 8 h ozone exposure. Adult animals demonstrated significant decreases in NADPH content (45%↓) and the reduced/oxidized ratio (38%↓), no decrease in $NADP^+$ concentration, and a marginal decrease in the sum of [NADPH] + [$NADP^+$]. This indicates clearly that 1.5 ppm O_3 for 8 h constitutes a marked oxidant stress with a significant effect on lung redox capacity in the normal, adult animal.

Aged rats demonstrated much larger, significant decreases in both NADPH and $NADP^+$ concentrations, their sum, and ratio. The largest response observed was in the concentration of

TABLE I

EFFECT OF OZONE EXPOSURE ON LUNG TRIPHOSPHONUCLEOTIDE CONCENTRATION AND BALANCE IN ADULT AND AGED RATS

	Adult		Aged	
	control [a]	O_3-exposed [b]	control [a]	O_3-exposed [b]
nmol NADPH per g wt. weight	8.4 ±1.1	4.6 ±1.0 [c] (45%↓)	6.3 ±0.8	1.7 ±0.1 [d] (73%↓)
nmol $NADP^+$ per g wt. weight	7.0 ±0.5	6.5 ±0.7 (7%↓)	7.4 ±0.6	3.7 ±1.2 [d] (50%↓)
[NADPH]+[$NADP^+$]	15.4 ±1.3	11.1 ±1.3 [e] (28%↓)	13.7 ±1.0	5.4 ±1.2 [d] (60%↓)
[NADPH]/[$NADP^+$]	1.20±0.15	0.75±0.15 [c] (38%↓)	0.94±0.16	0.36±0.05 [c] (62%↓)

[a] $N = 12$ for adult and aged controls. Data are expressed as mean ± S.E.M.
[b] $N = 6$ for adult and aged O_3-exposed (1.5 ppm O_3 for 8 h).
[c] $P < 0.05$ compared to controls; unpaired Student's t-test, two-tailed.
[d] $P < 0.01$ compared to controls; unpaired Student's t-test, two-tailed.
[e] $P = 0.06$ compared to controls; unpaired Student's t-test, two-tailed.

NADPH, which decreased 73% from its control value in aged animals. Perhaps more importantly, a pronounced shift occurred from the reduced to the oxidized form. In adults, O_3 exposure resulted in a 38% decrease in the [NADPH]/[$NADP^+$] ratio (1.20 : 0.75). In aged animals, not only was there a more marked decrease in the total triphosphonucleotides (60% compared to 28% in adult animals), but that remaining in the aged animals was predominately in the oxidized form (reduced/oxidized ratio decreased 62% compared to 38% for adult animals). These data indicate that there are age-related differences in the response of the lung to oxidant insult. At least three differences are an increased sensitivity to the loss of reduced triphosphonucleotide (NADPH), a decreased availability of total triphosphonucleotides (NADPH + $NADP^+$), and perhaps most importantly, those triphosphonucleotides that do remain after oxidant insult in the aged animal are predominately in the oxidized form (NADPH/$NADP^+ = 0.36$).

Given the importance of the numerous biochemical roles of NADPH, it would be attractive to speculate on the many possible physiological consequences of this age-related difference. However, these preliminary findings require more thorough examination before such speculations can be supported. Numerous reports in the literature, including our own, document biochemical and morphometric alterations in the adult lung following chronic exposure to concentrations of ozone both greater and less than 1.5 ppm. Interpretation of all such studies are compromised by the inability to separate the initial injury from the repair process. A thorough morphometric analysis of the lungs obtained in this study is currently underway. Preliminary observations support our hypothesis that this exposure protocol produces acute lung injury with minimal repair (Balis, J.U. and Montgomery, M.R., unpublished data). Also of immediate interest is similar information on lower concentrations of ozone to identify the threshold of this age-related effect. These studies are also underway.

Supported by Veterans Administration Medical Research funds and NIH Grants ES03340 and HL34793. The authors acknowledge the excellent technical assistance of Ms. Sandra Raska.

References

1 Hayflick, L. (1979) Fed. Proc. 38, 1847–1850
2 Rothstein, M. (ed.) (1982) Biochemical Approaches To Aging, Academic Press, New York
3 Hansford, R.G. (1983) Biochim. Biophys. Acta 726, 41–80
4 Thurlbeck, W.M. (1980) in Aging – Its Chemistry, (Dietz, A.A., ed.), pp. 114–131, American Association of Clinical Chemistry, Washington, DC
5 Klocke, R.A. (1977) in Handbook of the Biology of Aging, (Finch, C.E. and Hayflick, L., eds.), pp. 432–444, Litton Educational Publishing, New York

6 Traurig, H.H. (1976) Gerontologia 22, 419–427
7 Wilson, P.D. (1972) Gerontologia 18, 36–54
8 Azcuy, A., Anderson, A.E. and Foraker, A.G. (1962) Ann. Int. Med. 57, 1–17
9 U.S. Department of Health, Education and Welfare (1967) The Surgeon General's Report: The Health Consequences of Smoking, Public Health Service Publication No. 1696
10 Averbach, O., Hammond, E.C., Garfinkel, M.A. and Benante, C. (1972) Engl. J. Med. 286, 853–857
11 Jeffrey, E.H. and Mannering, G.J. (1979) Mol. Pharmacol. 15, 396–409
12 Montgomery, M.R., Anderson, R.E. and Mortenson, G.A. (1976) Lab. Anim. Sci. 26, 461–464
13 Slater, T.F. and Sawyer, B. (1962) nature 193, 454–456
14 Witschi, H., Kacew, S., Hirai, K.-I. and Cote, M. (1977) Chem.-Biol. Interactions 19, 143–160
15 Nisselbaum, J.S. and Green, S. (1969) Anal. Biochem. 27, 212–217