Cadmium-Induced Depression of the Respiratory Burst in Mouse Pulmonary Alveolar Macrophages, Peritoneal Macrophages and Polymorphonuclear Neutrophils [1]

Leland D. Loose, Jay B. Silkworth[2] and Diane Warrington

Institute of Comparative and Human Toxicology
Albany Medical College
Albany, New York 12208

Received October 1,1977

Summary

No profound alteration in the resting O_2 consumption of mouse pulmonary alveolar macrophages, polymorphonuclear neutrophils or peritoneal macrophages incubated in media containing either cadmium chloride or cadmium acetate was observed. However, when heat-killed P. aeruginosa, opsonized in autologous serum, were added to the cell suspension a significant depression in the respiratory burst accompanying the phagocytic event was manifested. The suppression of the respiratory burst appeared to be related to the concentration of cadmium. The possible alteration in the relationship between macrophage microtubule assembly and endocytosis is discussed.

I. Introduction

A concomitant of phagocytosis is an increase in oxygen utilization, most of which, if carried out under aerobic conditions, is not cytochrome linked; there is also an increase in lactate production and a stimulation of the direct oxidative pathway for glucose metabolism during phagocytosis (1). The metabolic events occurring during phagocytosis have been reported to be similar for the various phagocytic cell types (2). However, this similarity may be qualitative since distinct differences in the chemiluminescent activity of alveolar and peritoneal macrophages and polymorphonuclear leucocytes and the inhibition of the responses by superoxide dismutase and Na^+ benzoate following exposure of the cells to a phagocytizable particle have been shown (3).

1. Supported by National Institute of Environmental Health Science grant 2-P01-ES00226-10, and by a joint program between the Gesellschaft für Strahlen -und Umweltforschung mbH, Munich, Germany and the Institute of Comparative and Human Toxicology, Albany Medical College, Albany, New York 12208.

2. J.B. Silkworth is a NIEHS pre-doctoral trainee supported by grant ES00103-10.

ISSN 0006-291X

Macrophage dysfunction has been suggested to be a significant contributing factor in the heavy metal-induced impairment in host responses to endotoxin (4,5,6), bacteria (7) and particulate antigens (8,9). Since phagocytosis is a crucial step in the induction of the primary immune response (10) and in protection against bacteria (11), viruses (12) and protozoa (13) and since heavy metals alter in vivo host defense mechanisms, the present study was conducted to ascertain the influence of cadmium salts on phagocytosis vis a vis the determination of the respiratory burst accompanying the phagocytic event.

II. Materials and Methods

Alveolar and peritoneal macrophages were obtained from male Balb/c mice (18-20 g) by pulmonary lavage and by peritoneal lavage (4 days after an intraperitoneal (ip) injection of 1 ml of 6% Na^+ caseinate), respectively. Polymorphonuclear neutrophils were collected from the peritoneal cavity by saline lavage 18 hours following an ip injection of 1 ml of 6% Na^+ caseinate. The cells were collected from their respective lavage fluids by centrifugation at 300 g for 10 minutes and then resuspended in Hanks Balanced Salt Solution (pH 7.4) (Gibco, 402S) supplemented with 10% fetal calf serum. Total cell counts were obtained using hemocytometer technique and differential counts were used as an index of purity.

Oxygen consumption of the isolated cell preparations was measured using a Gilson recording oxygraph equipped with a Clarke O_2 electrode. All measurements were conducted at 37^0C. The cadmium salts were prepared in the cell suspension medium buffered at pH 7.4. The cell preparations were incubated with the Cd^{++} salts for 15 minutes during which time their resting O_2 consumption was determined. Phagocytosis was initiated by the addition of heat-killed Pseudomonas aeruginosa opsonized in autologous serum using a bacteria: cell ratio of 100:1. The O_2 consumption of the phagocytizing cells, in the presence of the Cd^{++} salts, was measured for 15 minutes. The respiratory burst observed following the addition of the bacteria was determined by the difference in slopes of the lines representing resting O_2 consumption versus the phagocytizing O_2 consumption.

Table I

The Effect of Cadmium Salts on Resting O_2 Consumption by Isolated Murine Phagocytic Cells[a,b]

	Resting Cells $\mu MO_2/10^6$ Cells/Hour		
Cadmium Chloride (mEq Cadmium/L)	Alveolar Macrophages	Polymorphonuclear Leucocytes	Peritoneal Macrophages
0	7.8 ± 0.4	118 ± 10.4	47.8 ± 5.8
3.6×10^{-3}	7.4 ± 0.4	130 ± 7.1	61.4 ± 11.1
3.6×10^{-2}	8.6 ± 1.0	73 ± 15.0	52.0 ± 9.5
3.6×10^{-1}	10.2 ± 0.6	107 ± 13.4	48.9 ± 11.9
3.6×10	8.7 ± 0.3	55 ± 23.0	43.0 ± 2.4
Cadmium Acetate (mEq Cadmium/L)			
0	5.5 ± 1.6	184 ± 30.7	62.8 ± 18.2
3.6×10^{-3}	4.2 ± 0.8	136 ± 25.4	66.8 ± 18.6
3.6×10^{-2}	4.6 ± 1.2	140 ± 16.8	59.5 ± 18.1
3.6×10^{-1}	5.6 ± 0.9	127 ± 21.6	46.9 ± 13.1
3.6×10	5.6 ± 0.9	91 ± 9.5*	38.7 ± 10.9

[a]Cadmium chloride and cadmium acetate were prepared in 0.9% NaCl. The cells were incubated with the cadmium salts for 15 minutes after which O_2 consumption was determined using a Clarke O_2 electrode.

[b]Data is presented as mean ± standard error with * indicating significance at $p < .05$; $n = 3$ in all groups.

All chemicals were of reagent grade and sterile plastic and siliconized glassware were used in all procedures. Data is presented as the mean ± standard error with an asterisk denoting significance at $p < .05$.

III. Results

Neither cadmium chloride nor cadmium acetate significantly altered the resting O_2 consumption of alveolar or peritoneal macrophages. The resting O_2 consumption of PMNs was suppressed only at the highest concentration of cadmium

acetate (Table I). However, when an opsonized phagocytizable particle, i.e., P. aeruginosa was presented to the cells, a profound depression of the respiratory burst was observed which appeared to be related to the concentration of the cadmium salts in the medium (Table II).

The respiratory burst, which is a concomitant of the phagocytic event, was significantly suppressed in alveolar macrophages and PMNs incubated with cadmium chloride and in alveolar macrophages and peritoneal macrophages incubated in cadmium acetate, both at concentrations of 3.6×10^{-2} mEq/1. A decrease in the respiratory burst of peritoneal macrophages incubated in $CdCl_2$ or PMNs incubated with media containing cadmium acetate was not observed until a concentration of 3.6×10^{-1} mEq/1 of Cd^{++} was reached.

Discussion

Previous investigations have demonstrated that the chemiluminescent response of PMNs is approximately 3 orders of magnitude greater than alveolar macrophages and 1 order of magnitude greater than peritoneal macrophages. Inhibition of chemiluminescence by superoxide dismutase was more profound in the PMNs than in either alveolar or peritoneal macrophages. When Na^+ benzoate was used to inhibit chemiluminescence, the least inhibition was observed in the alveolar macrophages (3). In agreement with the differences observed in chemiluminescent activity between the various phagocytic cell types, a similar difference in the resting O_2 consumption between PMNs, alveolar macrophages and peritoneal macrophages was demonstrated in the present study. The resting O_2 consumption of PMNs was 2X greater than peritoneal macrophages and 8X greater than alveolar macrophages. Cadmium had no influence on the resting O_2 consumption of the phagocytic cells (Table I) which is similar to results recently reported in which zinc ions were used (14).

However, when an opsonized phagocytizable particle, i.e. Pseudomonas aeruginosa was presented to the phagocytic cells which had either cadmium acetate or cadmium chloride in their incubation media, a depression in the respiratory burst, which appeared to be related to the Cd^{++} concentration, was manifested

Table II

The Effect of Cadmium Salts on O_2 Consumption During Phagocytosis by Isolated Murine Phagocytic Cells[a,b]

	% Δ in O_2 Consumption		
Cadmium Chloride (mEq Cadmium/L)	Alveolar Macrophages	Polymorphonuclear Leucocytes	Peritoneal Macrophages
0	29.2 ± 2.2	35 ± 4.8	27.7 ± 7.9
3.6×10^{-3}	16.8 ± 4.6	36 ± 0.0	14.4 ± 3.6
3.6×10^{-2}	-10.5 ± 6.7*	2 ± 0.0*	8.0 ± 0.9
3.6×10^{-1}	-29.2 ± 3.2*	-21 ± 7.3*	-16.6 ± 1.5*
3.6×10	-26.0 ± 5.3*	-44 ± 6.8*	-19.0 ± 1.2*
Cadmium Acetate (mEq Cadmium/L)			
0	30.5 ± 4.7	53 ± 10.0	17.7 ± 0.7
3.6×10^{-3}	19.3 ± 5.8	30 ± 2.5	11.1 ± 2.5
3.6×10^{-2}	10.8 ± 1.6*	33 ± 3.5	7.6 ± 1.5*
3.6×10^{-1}	-9.7 ± 6.9*	-2 ± 2.3*	-2.2 ± 6.5*
3.6×10	-20.2 ± 2.1*	-35 ± 1.2*	-15.8 ± 1.6*

[a]The cells were incubated with the cadmium salts for 15 minutes after which Pseudomonas aeruginosa (100 bacteria: 1 cell) was added to the cell suspension. The difference between the slopes of the lines for resting versus phagocytizing O_2 consumption is presented as the % change in O_2 consumption, i.e. the "respiratory burst".

[b]Data is presented as mean ± standard error with * indicating signficance at $p < .05$; n = 3-4 in all groups.

(Table II). Peritoneal macrophages were least sensitive to the $CdCl_2$ induced depression of the burst than PMNs or alveolar macrophages which is in agreement with the demonstration that peritioneal macrophages are also the least sensitive phagocytic cell type to the cytotoxic effects of $CdCl_2$ (15). Cadmium acetate suppressed the respiratory burst less in the PMNs than that which was observed in

the alveolar or peritoneal macrophages (Table II). A similar metal-induced impairment of the respiratory burst has been reported for rat peritoneal macrophages and granulocytes incubated with media containing $ZnCl_2$ (14).

The mechanism whereby Cd^{++} may impair phagocytosis, as measured in this study by the classical respiratory burst accompanying the phagocytic-event, may be associated with the known inhibitory action of Cd^{++} on the ATPases of myosin (16) and the ATPases of pulmonary alveolar macrophage cells and cell membranes (17). Inhibition, by Cd^{++}, of the myosin ATPases is a particularly attractive hypothesis since a microtubule assembly containing actin-myosin like proteins, which is regulated partly by ionized Ca^{++}, has been suggested to play an integral role in cellular endocytosis (18). Direct measurements of the influence of Cd^{++} on cellular microtubule assembly and function would be desirable.

The demonstration of an alteration in phagocytic activity by three distinct populations of phagocytic cells may, in part, explain the observed synergism between Cd^{++} and bacterial (19) susceptibility and the *in vivo* impairment in antibody formation (8,9).

References

1. Sbarra, A.J. and Karnovsky, M.L. (1959) J. Biol. Chem. 234, 1355-1362.
2. Kay, N.E. and Douglas, S.D. (1977) N.Y. State J. Med. 77, 327-340.
3. Allen, R.C. and Loose, L.D. (1976) Biochem. Biophys. Res. Comm. 69, 245-252.
4. Selye, H., Tuchweber, B. and Bertoli, L. (1966) J. Bacteriol. 91, 884-890.
5. Truscott, R.B. (1970) Can. J. Comp. Med. Vet. Sci. 34, 134-137.
6. Cook, J.A., Marconi, E.A. and DiLuzio, N.R. (1974) Toxicol. Appl. Pharmacol. 28, 292-297.
7. Hemphill, F.E., Kaeberle, M.L. and Buck, W.B. (1971) Science 172, 1031-1032.
8. Jones, R.H., Williams, R.L. and Jones, A.M. (1971) Proc. Soc. Exp. Biol. Med. 137, 1231-1236.
9. Koller, L.D. (1973) Am. J. Vet. Res. 34, 1457-1458.
10. Frei, P.C., Benacerraf, B. and Thorbecke, G.J. (1965) Proc. Natl. Acad. Sci. U.S.A. 53, 20-23.

11. Biozzi, G., Halpern, B.N., Benacerraf, B. and Stiffel, C. (1975) Physiopathology of the Reticuloendothelial System, p. 204, Blackwell Scientific Publications, Oxford, England.

12. Mims, C.A. (1964) Bacteriol. Rev. 28, 30-71.

13. Taliaferro, W.H. (1929) The Immunology of Parasitic Infections, Century Publishing Co., New York.

14. Chvapil, M., Stankova, L., Bernhard, D.S., Weldy, P.L., Carlson, E.C. and Campbell, J.B. (1977) Inf. Imm. 16, 367-373.

15. Loose, L.D., Silkworth, J.B. and Warrington, D. (1977) Bull. Environ. Contam Toxicol. (In press).

16. Lipkan, G.N. (1970) Farmakol. Toksikol. Respub. Mezhvedom., S.B. 5, 191-197.

17. Cross, C.E., Ibrahim, A.B., Ahmed, M. and Mustafa, M.G. (1970) Environ. Res. 3, 512-520.

18. Allison, A.C., Davies, P. and DePetris, S. (1971) Nature New Biol. 232, 153-155.

19. Cook, J.A., Hoffmann, E.O. and DiLuzio, N.R. (1975) Proc. Soc. Exp. Biol. Med. 150, 741-744.