Note

Depression of Phagocytic Activity of Human Polymorphonuclear Leukocytes by Methyl Linoleate Hydroperoxides

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Summary Methyl linoleate hydroperoxides (MLHPO), a model for lipid hydroperoxides, decreased fungicidal activity of human polymorphonuclear leukocytes (PMN) for fungi of *Candida albicans*. When PMN was cultured with MLHPO, phagocytosis to fungi was depressed. Degree of the depression was dependent on the concentration of MLHPO and the treatment time with MLHPO. These findings indicate that phagocytic activity of human PMN can be depressed by lipid hydroperoxides.

Key Words lipid peroxides, polymorphonuclear leukocytes, phagocytosis

Several studies have been reported on the nutritive problems associated with dietary lipid hydroperoxides, and it was proved that lipid hydroperoxides were absorbed and incorporated into several organs of animals after an oral administration (1). Furthermore, the formation of lipid hydroperoxides in animal tissues has been reported (2), and the presence of lipid hydroperoxides in human plasma has been proved using a chemiluminescence detection-high performance liquid chromatography system (3). To know the influence of these lipid hydroperoxides on animals, methyl linoleate hydroperoxides (MLHPO) or autoxidized soybean oil have been orally administered to mice, and it has been found that lipid hydroperoxides cause significant effect to the maturation of thymocytes (4,5). Other kinds of immunocompetent cells such as polymorphonuclear leukocytes (PMN) generate unstable reduction products of oxygen during phagocytosis. It has been observed that phagocytosis is attenuated by autoxidative damage to the cell membrane by these endogenously derived oxygen by-products (6). Therefore, it is thought that PMN would be affected by dietary lipid hydroperoxides and/or lipid hydroperoxides generated in tissues. In this study, we have shown that phagocytic activity of human PMN is depressed by lipid hydroperoxides.

MLHPO was prepared as described previously (7). The purified MLHPO has a peroxide value (PV) of 6,086 meq/kg. To isolate human PMN, 20 ml of

heparinized blood (10 U of heparin/ml) from healthy donors was mixed with 4 ml of 6% Dextran T 250 (Pharmacia, Sweden) and allowed to stand at 37°C for 45 min. Then 12 ml of the buffy coat was collected and diluted with 12 ml of RPMI-1640 (GIBCO Laboratories, NY); 8 ml of diluted buffy coat was then layered over 3 ml of Histopaque ($d=1.077$, Sigma Chemical Company, St. Louis) in 15 ml conical plastic centrifuge tubes. Centrifugation was done at $400\times g$ for 40 min. Pellets were washed once with RPMI-1640 and treated with Tris-buffered 0.84% ammonium chloride (pH 7.2) to lyse contaminating red blood cells. Treated cells were washed with RPMI-1640 and suspended in complete tissue culture medium [CTCM; 80% RPMI-1640, penicillin (100 U/ml), streptomycin (100 $\mu\text{g}/\text{ml}$), 10% (vol/vol) heat-inactivated fetal bovine serum (GIBCO Laboratories), and 10% (vol/vol) fresh autologous serum]. Wright-stained smears showed that the cells were 95% PMN. PMN were over 98% viable as assessed by the 0.4% erythrosine dye exclusion test. Cells were suspended to 5×10^6 cells/ml CTCM. For fungicidal assay, 100 μl of cell suspensions were placed in flat-bottom microtest plate wells and 100 μl CTCM were added to each well. MLHPO or methyl linoleate (ML; PV = 0.6 meq/kg) dissolved in dimethyl sulfoxide (DMSO) was added to the PMN suspensions. The final concentrations of MLHPO, ML and DMSO in culture solutions were 245 μM , 245 μM , and 128 mM, respectively. After 4 h of culturing, PMN were washed twice with CTCM and 200 μl of *C. albicans* [5,000 colony-forming units (CFU)/ml CTCM] was added. Cultures were incubated for 15, 30, 60, 120, 180 and 240 min at 37°C in 5% CO_2 -95% air. After incubation, well contents were removed and placed into 5 ml of distilled water, and each well was washed five times with 0.2 ml of distilled water. The culture contents and washings were diluted to 10 ml. After the diluted fluid was vigorously mixed, 1 ml of harvested material was plated on 5% sheep red blood cell trypticase soy agar plates, and CFU per culture were determined after 2 days at room temperature. The percent CFU was calculated by the formula: (experimental CFU divided by inoculum CFU) $\times 100$. For observation of phagocytosis, PMN (5×10^5) were cultured in 200 μl CTCM on microtest plate wells. MLHPO or ML dissolved in DMSO were added to the PMN suspensions. The final concentrations of MLHPO, ML and DMSO in culture solutions were 245 μM , 245 μM , and 128 mM, respectively. After 4 h of culturing (at 37°C in an atmosphere of humidified 5% CO_2 /95% air), cultured PMN were washed twice with CTCM. Then PMN (5×10^5) and *C. albicans* (15×10^5 CFU) were incubated in 200 μl of CTCM for 15, 30, 60, 120, 180, and 240 min at 37°C in 5% CO_2 -95% air. Cells were pelleted by centrifugation ($300\times g$, 5 min) and suspended in 50 μl of CTCM, and the phagocytosis was observed under a light microscope. About 150 PMNs were observed in each suspension. Percent of phagocytosis was determined by the formula: (number of PMN with one ingested fungi or more divided by the number of PMN examined) $\times 100$. Viability of PMN after the treatment with DMSO, ML, or MLHPO was assessed by the 0.4% erythrosine dye exclusion test.

In cases of untreated, DMSO-treated, and ML-treated PMN, CFU of *C.*

albicans were decreased to around 20, 10 and 5% after 120, 180 and 240 min of incubations with fungi, respectively (Fig. 1). By contrast, CFU was over 80% in the MLHPO-treated PMN throughout experimental duration. This result shows fungicidal activity of human PMN was depressed by the culturing with MLHPO before the incubation with fungi. By the 4 h of culturing with DMSO, ML or MLHPO, PMN were over 95% viable, respectively. After 15 min of incubation with fungi, the phagocytosis was already 86, 95, and 86% in untreated, DMSO-treated, and ML-treated PMN, respectively (Fig. 2). In the case of MLHPO-treated PMN, the phagocytosis was depressed throughout experimental duration. Degree of the depression was dependent on the concentration of MLHPO. Figure 3 shows the influence of the culturing time with MLHPO on phagocytic activity of human PMN. The degree of depression in phagocytosis tended to be dependent on the culturing time with MLHPO also. From these results, it should be assumed that MLHPO-treated PMN could not kill the fungi because of the impairment of the phagocytosis by MLHPO.

This study shows that phagocytic activity of human PMN is depressed by lipid hydroperoxides.

Fig. 1

Fig. 1. Effect of methyl linoleate hydroperoxides (MLHPO) on fungicidal activity of human PMN. PMN were cultured with MLHPO or ML dissolved in DMSO for 4 h. Then cultured PMN were washed and incubated with *C. albicans*. Untreated, ○; DMSO (128 mM)-treated, △; ML (245 μ M)-treated, ●; MLHPO (245 μ M)-treated, ▲. Values represent mean $\pm$ SD of quadruplicate cultures.

Fig. 2

Fig. 2. Effect of methyl linoleate hydroperoxides (MLHPO) on phagocytosis of human PMN. PMN were cultured with graded concentrations of MLHPO or ML dissolved in DMSO for 4 h. Then cultured PMN were washed and incubated with *C. albicans*. Untreated PMN, ○; DMSO (128 mM)-treated PMN, □; ML (245 μ M)-treated PMN, ■; MLHPO-treated PMN: ▼, 25; △, 123; ●, 184; ▲, 245 μ M. Values represent mean $\pm$ SD of triplicate cultures.

Fig. 3. Effect of culturing time with MLHPO on phagocytosis of human PMN. PMN were cultured with MLHPO dissolved in DMSO for graded times (5, 15, 30, 60, 120, and 180 min). Then cultured PMN were washed and incubated with *C. albicans* for 2 h. MLHPO: ○, 184; ●, 245 μ M. Values represent mean $\pm$ SD of triplicate cultures.

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