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Lipoic Acid Suppression of Neutrophil Respiratory Burst: Effect of NADPH

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ABSTRACT

Lipoic acid (LA) and its reduced product dihydrolipoic acid (DHLA) are potent antioxidants with capacity to scavenge reactive oxygen species (ROS) and recycle endogenous antioxidants. LA may increase cellular glutathione (GSH), an antioxidant lacking in the lung's epithelial lining fluid in lung disorders such as idiopathic pulmonary fibrosis (IPF). Neutrophils (PMN) are key innate responders and are pivotal in clearing bacterial infection, therefore it is crucial to understand the impact LA may have on their function. Circulating neutrophils were isolated from healthy volunteers and pretreated with LA or diluent. Cells were subsequently activated with phorbol 12-myristate 13-acetate (PMA, 100 ng/ml) to induce ROS production. SOD-inhibitable reduction of acetylated cytochrome c demonstrated the PMA-dependent respiratory burst was suppressed by LA. Oxygen consumption also was diminished when PMA-stimulated cells were pretreated with LA. PMN respiratory burst was partially restored by addition of NADPH but not other pyridine nucleotides. LA did not inhibit glucose-6-phosphate dehydrogenase activity of PMN. These data together suggest that the reduction of LA to DHLA using cellular NADPH may limit the capacity of the PMN NADPH oxidase to produce superoxide. Further studies will be required to determine if LA can diminish excessive superoxide produced by PMN and/or alveolar macrophages in IPF or relevant disease models *in vivo*. *Antioxid. Redox Signal*. 10, 277–285.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic and typically fatal inflammatory disorder characterized by elevated levels of lymphocytes, alveolar macrophages, and neutrophils (PMN) within the lower respiratory tract (12, 40, 52). Although the primary cell present in IPF is the lymphocyte, reported levels of PMN range from 7 to 23% in lung bronchoalveolar lavage fluid (BAL) from IPF patients (13, 31, 34, 40). Neutrophils are prominent early in IPF disease models such as that caused by bleomycin and, indeed, IPF patients may not reach the care of a pulmonologist until after the acute phase of inflammation.

Activated PMN increase the oxidative burden in the lung by releasing excessive reactive free radicals, which ultimately contributes to tissue damage (11, 51). Specifically, in IPF, reactive oxygen species (ROS) production by BAL cells, both basal and stimulated, was 7- to 10-fold greater than in nondiseased controls (51) and, in that study, alveolar macrophages appeared to be the primary source of ROS. However, the cellular source(s) of oxidative stress in IPF are controversial. Others found that methionine oxidation was increased in BAL fluid from nonsmoking IPF patients, and that this was strongly correlated with neutrophil, but not alveolar macrophage, counts in BAL (28). In addition to IPF, methionine oxidation also was elevated and correlated positively with BAL neutrophils in pulmonary fibrosis in association with collagen vascular diseases (5). Oxidant injury to lung also was evidenced by protein oxidation in BALF of nonsmoking IPF and sarcoidosis patients (24). Interleukin-8 (IL-8) mRNA and protein also were elevated in IPF versus controls, and these correlated with BAL neutrophils (27)

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and disease severity (9). IL-8 distribution (58), BAL percentage, and absolute neutrophil numbers also correlated with extent of lobar involvement in IPF patients. BAL neutrophil and eosinophil products, myeloperoxidase, and eosinophil cationic protein, respectively, also tracked clinical improvements and deteriorations after therapy (41), whereas neutrophil elastase and its inhibitors appeared to play a lesser role in IPF compared to other pulmonary disease processes such as bronchiectasis (47). In summary, free radicals generated by infiltrating inflammatory cells are believed to contribute to injury, faulty repair, and subsequent fibrosis in the lung.

The oxidant—antioxidant imbalance is further exacerbated by a deficiency in epithelial lining fluid (ELF) glutathione (GSH), the major small molecular weight extracellular lung antioxidant (8, 44). Thiols such as GSH and its precursor *N*-acetyl cysteine (NAC) have been utilized to improve the oxidant—antioxidant imbalance and reduced levels of GSH in the ELF of IPF patients with improvements in some parameters. Aerosolized GSH resulted in a transient increase in ELF GSH and decreased alveolar macrophage superoxide production (6). NAC, in conjunction with prednisone and azathioprine, has been shown to maintain lung vital capacity and single-breath carbon monoxide diffusing capacity in IPF patients (6, 14, 16). α-Lipoic acid (LA), a dithiol compound derived from octanoic acid, exists endogenously as lipoamide and plays an essential role as a cofactor in mitochondrial dehydrogenase reactions including pyruvate, α-ketoglutarate, and branched chain α-ketoacids (33). LA is capable of scavenging the hydroxyl radical and hypochlorous acid while its reduced form dihydrolipoic acid (DHLA) has the capacity to scavenge superoxide and peroxyl radicals as well as hypochlorous acid (21, 48, 53, 54). In addition, the LA/DHLA redox couple has a low potential of -320 mV, making it a powerful reductant. This reducing capacity allows DHLA to nonenzymatically regenerate GSH and vitamin C (3, 15). Thus, the capacity of the LA/DHLA couple to scavenge radicals and regenerate endogenous antioxidants suggests potential benefit for treatment of IPF.

Previous studies have demonstrated that LA readily enters mammalian cells, is reduced to DHLA, and released extracellularly (17). The reduction of LA to DHLA requires NADH in the case of the mitochondrial lipoamide dehydrogenase or NADPH for glutathione reductase and thioredoxin reductase (2, 18). Oral administration of LA results in measurable levels in the plasma; hence blood cells including the neutrophil can be a target for its action (56). Modes of delivery that target the lung, which could further increase tissue LA levels (*e.g.*, aerosol), have not been evaluated. As cells reduce LA to DHLA, it is also feasible that DHLA released from lung epithelial cells would come into contact with PMN in the IPF lung as well. As such, it is important to understand the impact LA treatment will have on PMN.

PMN are terminally differentiated granular phagocytes that play an essential role in the body's innate defense against pathogens and have a range of bactericidal products. The arsenal of the PMN includes superoxide and hypochlorous acid, bactericidal peptides, and various enzymes including elastase and myeloperoxidase, the latter of which comprises 5% of PMN total protein (22). The shortened lifespan of neutrophils relative to other cell types may be influenced by their lower levels of antioxidant systems including GSH and thioredoxin, as well as lower levels of antioxidants enzyme including manganese superoxide dismutase and glutathione peroxidase (23). Various factors have been reported to extend PMN lifespan in IPF. For example, anti-apoptotic Bcl-2 levels are increased in IPF PMN and eosinophils. IL-1β levels are also elevated in macrophages from IPF patients, which can prolong the survival of PMNs up to 72 h (10, 30, 59).

PMN ROS production through the respiratory burst requires the assembly of the NADPH oxidase. The membrane associated catalytic core includes $p22^{phox}$ and $gp91^{phox}$ (also known as Nox2) which serve as a docking site when cytosolic $p40^{phox}$, $p47^{phox}$, $p67^{phox}$, and Rac (Rac2 in PMN, Rac1 in macrophages) proteins translocate to form the functional NADPH oxidase (20, 36). Once assembled, the oxidase performs the one-electron reduction of oxygen utilizing NADPH as a cofactor to produce superoxide and subsequent radical metabolites including H_2O_2 and hypochlorous acid. NADPH needed as substrate for the oxidase is produced through the pentose phosphate pathway (PPP), with $NADP^+$ as the rate-limiting substrate and glucose-6-phosphate dehydrogenase as the rate-limiting enzyme (4). The production of ROS by PMN is an essential bactericidal process but can result in host tissue damage when out of balance with antioxidant levels, as is hypothesized in IPF.

In isolated peripheral human neutrophils, *N*-acetylcysteine has the ability to decrease luminol chemiluminescence, indicating decreased ROS production (50). The monothiol nacystelyn, a lysine salt derivative of NAC, also reduces ROS production assessed with luminol- and lucigenin-enhanced chemiluminescence (ECL) (35). However, no studies to date have reported the impact a dithiol such as dihydrolipoic acid (DHLA), or its precursor LA, will have on the production of ROS in PMN. We hypothesized that exposure to LA would result in decreased ROS production and that utilization of NADPH in the conversion of LA to DHLA could compete with the NADPH required for the one electron reduction of oxygen to superoxide by the NADPH oxidase, reducing ROS production of PMN. This could be beneficial to IPF patients in reducing oxidative stress.

METHODS

Neutrophil isolation

Protocols were reviewed and approved by the NJMRC Institutional Review Board. Human neutrophils were purified from whole blood by Percoll gradient as described by Haslett *et al*. (19). Preparations utilized in these experiments exhibited a PMN purity of $>95\%$ with $<2\%$ eosinophils, and cell viability $>98\%$ as measured by trypan blue exclusion. PMN were then resuspended in RPMI 1640 with 1% BSA in 1.5 ml Eppendorf tubes at 37°C (5% CO_2).

Reagents

A 20 m*M* stock solution of LA was prepared in a solution containing 1 ml water treated with chelex resin (Chelex water prepared according to manufacturer's instructions; BioRad, Hercules, CA) containing 5% bicarbonate, 200 μl 10x PBS, and 800 μl chelex water. The pH was adjusted to 8.0 with 3 *N* hy-

drochloric acid. Prereduced recombinant human thioredoxin (Trx) was a generous gift of Syngenta (London, UK). Phorbol 12-myristate 13-acetate (PMA) (Sigma P1585, St. Louis, MO) was resuspended in DMSO at a concentration of 1 mg/ml, with final concentration of 100 ng/ml in all assays. NADPH (Sigma N1630), NADH (Sigma N8129), nicotinamide (Sigma N3376), and nicotinic acid (Sigma N4126) were all at a final concentration of 1 m*M*. 6-aminonicotinamide (Sigma A68203) was at a final concentration of 10 m*M*.

Measurement of $O_2^{-\cdot}$ release from neutrophils

Generation of extracellular $O_2^{-\cdot}$ release from cells in suspension was measured utilizing the SOD-inhibitable reduction of cytochrome c by the method of Crapo *et al*. (10). A concentration of 0.1 m*M* ferric acetylated cytochrome c (Sigma C4186, ~60% lysine residues acetylated) was determined using the reduction of dithionite at 550 nm utilizing the extinction coefficient of 2.1×10^4 mol/L.cm. To avoid loss of the detection molecule in reactions with DHLA, cells were pretreated with LA or Trx for 30 min, followed by a wash step with 50 m*M* phosphate buffer. Cells were then resuspended in cytochrome c buffer (50 m*M* potassium buffer with 2 g/L glucose added) containing the predetermined amount of cytochrome c and the absorbance read in a spectrophotometer at 550 nm for 15 min. Cytochrome c reduced (nmols per min per 10^6 neutrophils) was determined utilizing the extinction coefficient of 2.1×10^4 mol/L.cm.

Extracellular oxygen consumption

Prior to experimentation, O_2 saturation values were determined for RPMI 1640 with 1% BSA utilizing the phenazine methosulfate-NADH method as described by Robinson and Cooper (45). Oxygen concentration was measured as described by Ahmad *et al*. (1). Briefly, O_2 concentration in the extracellular medium was determined utilizing a custom-built six-chamber respirometer. Each chamber consisted of a glass water-jacketed cell (Gilson Medical Electronics, Middleton, WI) fitted with a Clark-style polarographic O_2 electrode (model 5331, Yellow Springs Instruments, Yellow Springs, OH). The chambers were arranged over a multiposition electromagnetic stir plate (Cole—Parmer, Vernon Hills, IL) and placed in a tissue culture incubator to maintain 37°C. Six electrodes were connected to the Chemical Microsensor II (Diamond General Development, Ann Arbor, MI) through a 10-channel multiplexer. Channel selection and data acquisition were obtained through software written in LabVIEW (National Instruments, Austin, TX). The water-jacketed chambers were equilibrated with 1.9 ml RMPI +1% BSA for 30 min prior to experimentation. PMN were suspended in 100 ìl media and loaded into a water-jacketed cell; O_2 concentration was measured for 30 min. The slopes representing O_2 consumption were used to calculate nmol oxygen consumed per minute per 10^6 PMN.

Glucose-6-phosphate dehydrogenase activity assay

Freshly isolated neutrophils were suspended at the concentration of 2×10^6 per ml and treated with 500 μM lipoic acid for 30 min. The assay was performed based on the methods of Lohr and Waller (26). Briefly, after treatment, cells were centrifuged, the supernatant removed, and the cells gently resuspended in 200 μl Tyrode solution (137 m*M* NaCl, 2.7 m*M* KCl, 500 μM $MgCl_2 \cdot 6H_2O$, 320 μM $Na_2HPO_4 \cdot 2H_2O$. 5.5 m*M* glucose, and 12 m*M* $NaHCO_3$), 80 μl deionized H_2O, 80 μl triethanolamine buffer (50 m*M* triethanolamine HCl, 5.4 m*M* EDTA, pH 7.5), and 40 μl saturated digitonin solution (10 g/L). Cell solution then stood in the refrigerator for 1 h, followed by 15 mincentrifugation (3,000 *g* at 4°C). Supernatant was stored at −80°C until assay. For the assay, 67 μl of sample was loaded in a cuvette with 900 μl triethanolamine buffer and 16.7 μl 30 m*M* $NADP^+$ solution, mixed and incubated for 5 min at 25°C. After incubation, 16.7 μl 40 m*M* glucose-6-phosphate solution was added to initiate the reaction. The reaction was followed kinetically for 10 min to determine the volume activity by measuring the increase in NADPH spectrophotometrically at 340 nm.

Myeloperoxidase EIA assay

Myeloperoxidase (MPO) levels were determined from the supernatants of neutrophils using the TiterZyme Enzyme Immunometric Assay (EIA) (Assay Designs, Ann Arbor, MI). PMN were either left untreated, treated with 500 μM LA, 100 ng/ml PMA alone, or the combination of LA+PMA. Supernatants from 1×10^6 PMN were stored at −80°C for later use in the assay. Data was expressed in ng myeloperoxidase /ml of supernatant.

Data analysis

Data are presented as mean ± SEM. Comparisons between multiple groups were made utilizing one-way analysis of variance (ANOVA) and Bonferroni's Test for *post hoc* analysis (Graph Pad Prism v. 4.0c; San Diego, CA) with significance attained at $p \leq 0.05$.

RESULTS

Effect of LA and Trx on PMN ROS production

To determine if LA or Trx treatment altered the respiratory burst of the neutrophil, we measured extracellular $O_2^{-\cdot}$ release utilizing acetylated cytochrome c. Neutrophils were exposed to LA or Trx for 30 min, followed by a wash step to remove any extracellular thiols that could interact with the detector molecule cytochrome c. Figure 1 shows that LA exposure without the addition of PMA results in no respiratory burst and mirrors that of untreated cells. Exposure to PMA alone resulted in a robust respiratory burst maximal 6 min after the stimulus was added. Area under the curve (AUC) calculations demonstrated that pretreatment with 10, 50, or 100 μM concentrations of LA did not result in significant decrease in ROS production in the presence of PMA. However, production of ROS due to PMN stimulation by PMA was suppressed as a result of pretreatment with 500 μM LA as compared to PMA alone by AUC calculations (5.385 ± 0.388 *vs.* 7.738 ± 0.481, respectively, $p <$ 0.05). When cells were pretreated with Trx followed by PMA,

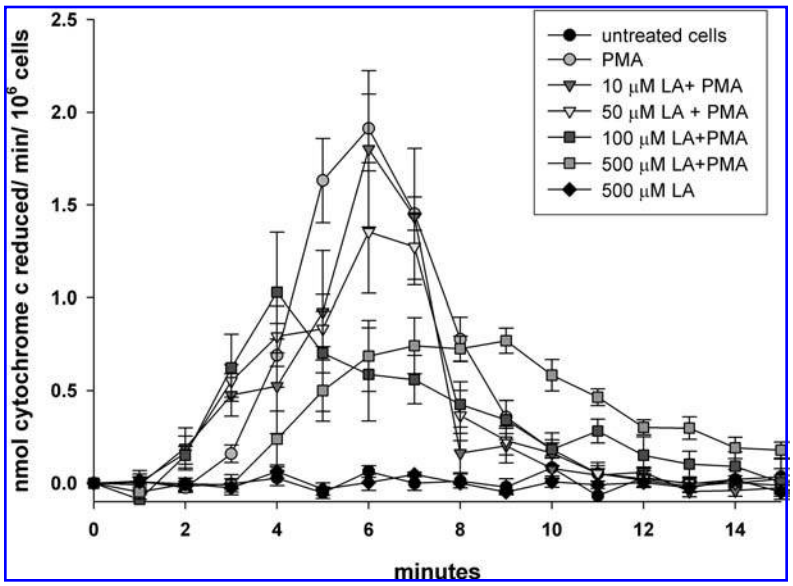

FIG. 1. Effect of LA on respiratory burst as measured by cytochrome c reduction. Thirty minutes of LA pretreatment followed by PMA results in a reduction in the respiratory burst at levels >100 m*M* as compared to PMA alone. Treatment with LA alone does not result in a measurable respiratory burst. $n = 6$, ± SEM.

there was no significant change in respiratory burst as compared to PMA alone (Fig, 2).

Effect of exogenous pyridine nucleotides or precursors

To determine whether NADPH restores respiratory burst activity after exposure to LA, we measured PMN reduction of acetylated cytochrome c upon PMA exposure following prior PMN incubation with exogenous pyridine nucleotides. Neutrophils were pretreated with LA and nicotinic acid, nicotinamide, NADH, or NADPH. After 30 min, neutrophils were washed and the respiratory burst was measured using acetylated cytochrome c reduction. Figure 3A demonstrates that exogenous NADPH partially restores the loss of respiratory burst measured by cytochrome c reduction, while the other compounds did not result in respiratory burst recovery. AUC calculations show the addition of 1 m*M* NADPH with 500 μM LA pretreatment prior to PMA stimulation resulted in a significant increase in respiratory burst as compared to 500 μM LA + PMA (7.949 ± 0.363 *vs.* 4.113 ± 0.5452, respectively, $p < 0.05$). Figure 3B shows that the addition of exogenous NADPH alone did not alter the respiratory burst of untreated cells. The addition of NADPH pretreatment prior to PMA alone also did not increase respiratory burst, indicating that the exogenous NADPH itself is not the cause for the increase in respiratory burst apparent following LA and NADPH pretreatment.

Measurement of oxygen consumption

Diminution of the respiratory burst can be due to lack of oxygen, lack of NADPH, or lack of assembly or dysfunction of an assembled NADPH oxidase. Since LA diminishes superoxide production as measured by the cytochrome c assay, a reason-

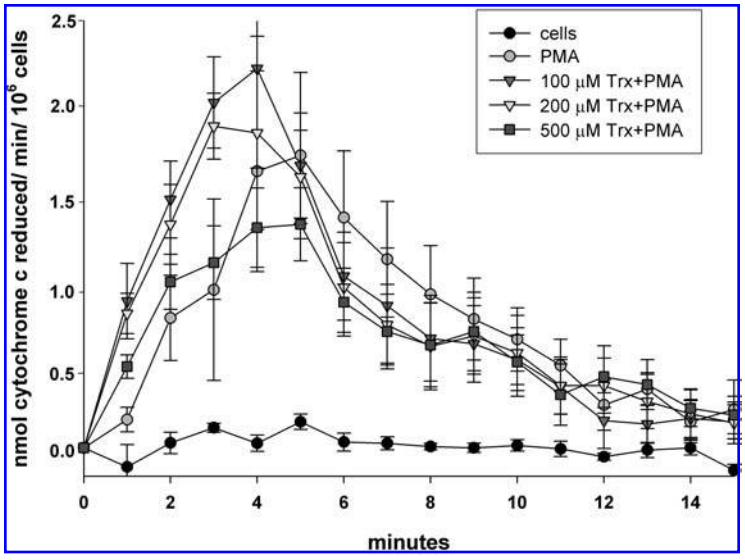

FIG. 2. SOD-inhibitable cytochrome c reduction by human neutrophils stimulated by PMA. Effect of Trx. Thirty minutes of Trx pretreatment did not result in the suppression of respiratory burst seen with LA. $n = 6$, ± SEM.

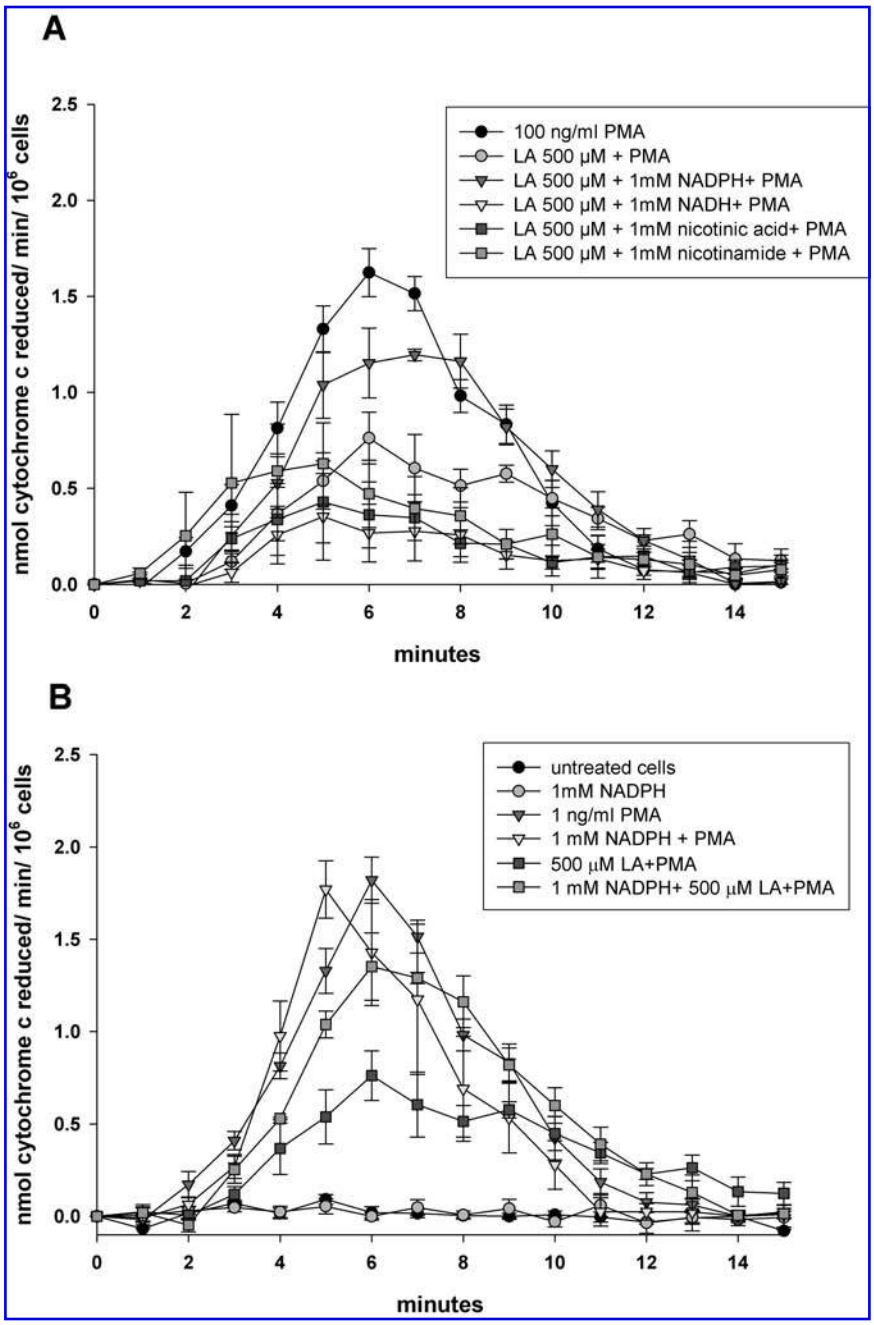

FIG. 3. Effect of NADPH on LA suppression of PMA-induced respiratory burst. (**A**) demonstrates that with the addition of pyridine nucleotides NADH, NADPH, or the precursors nicotinic acid or nicotinamide, that only NADPH addition helped to restore the LA-induced suppression of the respiratory burst. (**B**) shows that NADPH alone does not result in a respiratory burst; NADPH with PMA results in respiratory burst levels similar to that seen with PMA alone, indicating no enhancement. When cells are pretreated with NADPH and LA and subsequently stimulated with PMA, the suppression of respiratory burst is partially recovered. $n = 5$, $\pm$ SEM.

able method to differentiate between an NADPH oxidase deficiency and lack of substrate is to determine if oxygen consumption is also altered per the following equation:

$$2O_2 + \text{NADPH} \rightarrow 2O_2^{-\cdot} + \text{NADP}^+ + \text{H}^+$$

Similar to the pattern seen in the cytochrome c assay, Fig. 4 demonstrates that pretreatment for 30 min with LA led to a decrease in oxygen consumption when the cells were thereafter stimulated with PMA as compared to PMA treatment alone. Addition of exogenous NADPH to the LA pretreatment step resulted in a significant increase in oxygen consumption as compared to LA+PMA alone. These findings indicate decreased availability of NADPH for oxidase activity, rather than a decrease in the activity of the oxidase itself.

PMN glucose-6-phosphate dehydrogenase activity

To determine if the apparent lack of NADPH available for the respiratory burst was due to impaired pentose phosphate pathway (PPP) activity, the enzyme activity of glucose-6-phosphate dehydrogenase, the first enzyme in the pathway, was assayed. Figure 5 demonstrates that there was no difference in enzyme activity when cells were exposed to LA as compared to untreated cells. The addition of 6-aminonicotinamide, an inhibitor of PPP activity, significantly suppressed

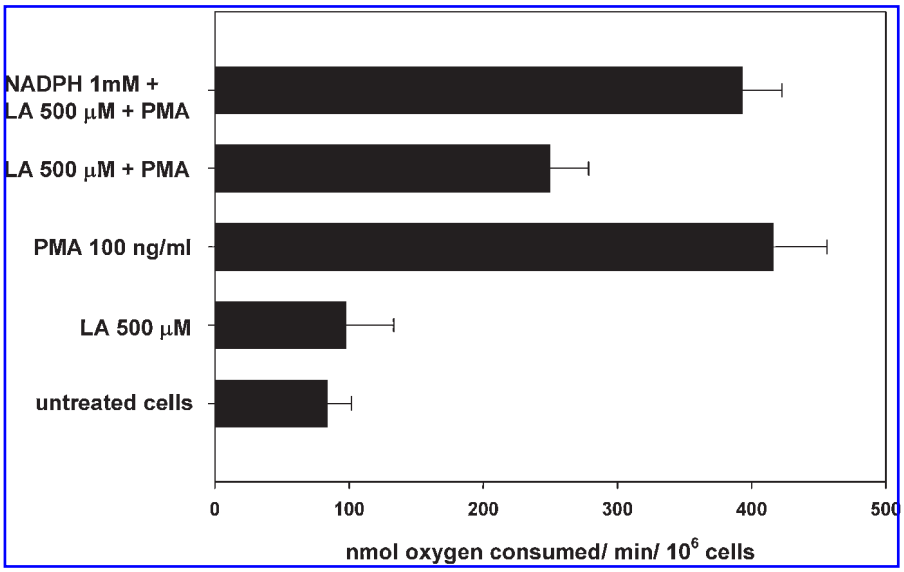

FIG. 4. Effect of LA on oxygen consumption in PMA-stimulated human neutrophils. PMA stimulus alone results in a fourfold increase in oxygen consumption while pretreatment with LA followed by PMA stimulus results in significantly decreased oxygen consumption as compared to PMA alone ($p < 0.01$). Pretreatment with NADPH and LA followed by PMA stimulus significantly increased oxygen consumption over LA+PMA alone ($p < 0.05$). $n = 7$, ± SEM.

G-6-PD activity as compared to LA alone, PMA alone, or the combination of LA+PMA, indicating the ability of the assay to measure differences activity in the presence of a potent inhibitor.

Myeloperoxidase protein levels

One mechanism known to alter NADPH is the electrophilic addition of HOCl to the C5–C6 double bond of the pyridine ring (42). As PMN produce HOCl in the presence of MPO, we sought to determine if LA treatment would result in an increase in myeloperoxidase (MPO) release that could enhance enzymatic conversion of H_2O_2 to hypochlorous acid once the PMN NADPH oxidase is activated. Studies demonstrated no increase in MPO as a result of exposure to LA (data not shown).

DISCUSSION

These studies demonstrated that exposure to LA prior to stimulation with PMA results in decreased respiratory burst in purified human PMN as measured by the reduction of acetylated cytochrome c. This was a dose-dependent effect with 100 μM approaching a significant decline in AUC and 500 μM attaining significance (Fig. 1). In contrast, pretreatment with the protein dithiol Trx did not result in a decreased respiratory burst (Fig. 2). LA is a small compound that easily passes the cell membrane while Trx is a larger protein dithiol that will remain primarily extracellular, suggesting LA's ability to enter the cell plays a role in respiratory burst suppression.

Decreased respiratory burst in PMN can imply lack of substrate or impaired NADPH oxidase activation. Our data demonstrate that when PMN are pretreated with LA and subse-

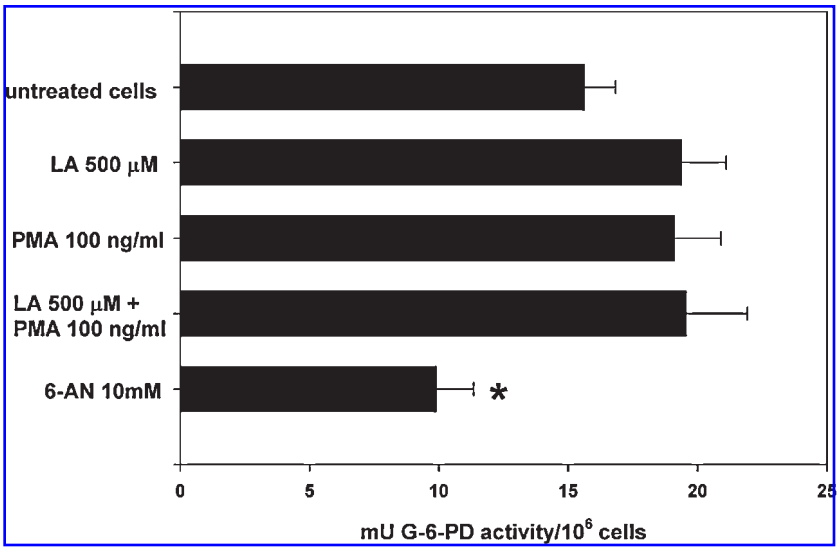

FIG. 5. Effect of LA exposure on glucose-6-phosphate dehydrogenase activity in human neutrophils. Exposure to LA, PMA, or LA+PMA did not result in a significant increase in enzyme activity. Pretreatment with 6-AN resulted in a significant suppression of enzyme activity as compared to all groups ($p < 0.05$). $n = 5$, ± SEM.

quently activated with PMA, there is a decline in oxygen consumption. This reaction is catalyzed by the NADPH oxidase, as shown below.

$$2O_2 + NADPH \rightarrow 2O_2^{-\cdot} + NADP^+ + H^+$$

These data combined suggested either impaired assembly or function of the NADPH oxidase. We observed that exogenous NADPH was effective in restoring respiratory burst activity of PMN, as measured by both acetylated cytochrome c reduction and oxygen consumption. This demonstrates the NADPH oxidase is functional and capable of assembly in the presence of LA. Since LA consumes NADPH for its reduction to DHLA, we propose that pretreatment with LA results in depletion of NADPH stores. As a result, PMN respiratory burst is suppressed.

NADPH oxidases expressed in phagocytes such as PMN only become activated once cells are exposed to pathogens or proinflammatory mediators. In contrast, NADPH oxidases (Nox) in other cell types are already assembled and only required NADPH to initiate respiratory burst (25). Studies have shown that exogenous NADPH is capable of stimulating superoxide production in intact vascular ring segments (38), isolated mouse aortic segments (49), as well as isolated sperm (46). As demonstrated by our studies, the NADPH oxidase was not producing significant ROS in the presence of NADPH alone; only the addition of PMA resulted in a respiratory burst as would be typical for stimulated PMN. As the only known NADPH binding site on the phagocytic NADPH oxidase is cytosolic, it is possible that exogenous NADPH is taken up by PMN by an as yet undetermined mechanism. Increased cytosolic levels would then be available for the respiratory burst once the oxidase is assembled. Second, it is possible that there is an alternative, extracellular NADPH binding site. This has been proposed for vascular Nox isoforms, but not yet confirmed. Alternately, as PMN burst in the presence of PMA, cells are lysing. The formerly cytosolic NADPH binding site could then be accessible for binding exogenous NADPH, resulting in enhanced NADPH utilization and increased ROS production.

In mammalian cells, NADPH is produced by several enzymes including glucose-6-phosphate dehydrogenase, the rate limiting enzyme in the pentose phosphate pathway (PPP) (39, 57). Glucose-6-phosphate dehydrogenase is functional in PMN pretreated with LA, indicating that the initial step in the PPP is functional. It is possible that alterations of NADP+, the rate limiting substrate in the PPP, also could occur as a result of exposure to LA, although this is yet to be determined.

One known mechanism of NADPH destruction is the electrophilic addition of HOCl to the C5═C6 double bond of the pyridine ring (42). PMN release MPO, which catalyzes formation of HOCl once the NADPH oxidase is activated and H_2O_2 is available. If LA pretreatment resulted in release of MPO and subsequent activation produced extracellular HOCl that readily enters cells, this could result in destruction of NADPH. This was not the case as MPO release was not enhanced as a result of pretreatment with LA (data not shown).

In inflammatory disease states such as IPF, the majority of ROS produced in the inflamed lung are likely produced by increased numbers of inflammatory cells including PMN (22). Treatments for IPF have been targeted at decreasing that oxidant burden and have included GSH and NAC. Clinical problems have been noted with GSH administration, for example, aerosolized GSH resulted in bronchoconstriction in asthmatic patients. This may have been due to increased glutathione disulfide (GSSG) formation due to the increased oxidative burden or possibly the acidic pH of the compound (29). Formation of GSSG was also noted in IPF patients, which may simply reflect the increased oxidative stress present in the IPF lung (6). NAC is a cyste(i)ne donor that can be utilized as a precursor for GSH. Once absorbed through the gut, it is deacetylated to cysteine (43). NAC has been used therapeutically to increase levels of GSH as well as decrease inflammation with varying efficacy in different respiratory disorders (5, 7, 31, 32). In contrast to the monothiols NAC and GSH, dithiols such as the LA/DHLA couple have markedly greater redox potentials and could have enhanced capacity to alleviate oxidative stress in IPF patients. LA has no effect on $O_2^{-\cdot}$, but has been shown to scavenge HOCl, $OH^{\cdot}$, and $^1O_2$; DHLA is capable of scavenging $O_2^{-\cdot}$, HOCl, $OH^{\cdot}$, but has mixed results with $^1O_2$ (37). Combined, the LA/DHLA couple could be more effective in scavenging ROS than either GSH or NAC.

The overall significance of these findings will depend on LA concentration in various lung compartments (intravascular, interstitial fluid, alveolus, airways) and route of delivery. Although LA pharmacokinetics have been determined in the plasma in humans with oral dosing, levels in lung lavage fluid have yet to be determined (55). Future studies will be necessary to determine levels of LA in the lung as a result of oral dosing as well as whether suppression of ROS production occurs *in vivo* at achievable doses. Alternate routes of delivery (*e.g.*, inhaled) also have yet to be explored. LA may have potential for treatment in IPF due to its ability to suppress ROS production by inflammatory cells as shown in this study.

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ABBREVIATIONS

DHLA, dihydrolipoic acid; GSH, glutathione; IPF, idiopathic pulmonary fibrosis; LA, lipoic acid; PMA, phorbol myristate acetate; PMN, polymorphonuclear cells; PPP, pentose phosphate pathway.

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