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Association of changes in lysophosphatidylcholine metabolism and in microsomal membrane lipid composition to the pulmonary injury induced by oleic acid

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Alterations in the lipid composition of lung microsomal membranes occur in oleic acid-induced respiratory distress. The marked decrease in the phosphatidylcholine/lysophosphatidylcholine molar ratio could be related with an altered metabolism of lysophosphatidylcholine in these membranes. Results revealed that the activity of phospholipase A increased whereas that of acyl-CoA:lysophosphatidylcholine acyltransferase decreased. Microsomal lysophospholipase activity remained unchanged. On the other hand, the microsomal enzyme system involved in the de novo synthesis of diacylglycerol was impaired, and cholinephosphotransferase activity was lowered. These changes in the activity of some membrane-bound enzymes were not caused by changes in the membrane lipid fluidity since lipid structural order parameter (S_{DPH}) did not change and neither did the major factors on which the fluidity depends. The possible significance of microsomal lipid alterations in the pathogenesis of respiratory distress induced by oleic acid is discussed.

Introduction

The administration of oleic acid has been widely used as a model of Adult Respiratory Distress Syndrome (ARDS) because it reproduces the tachypnea, hypoxemia, loss of lung compliance and diffuse bilateral pulmonary infiltrates of ARDS [1]. Elevation of circulating free fatty acid concentrations may occur in many clinical states related with this syndrome such as long bone trauma, severe burns, trauma-induced epinephrine release and others [2].

The basic pathological process in both the clinical disorder and in oleic acid injury affects the alveolar epithelium and the pulmonary microvascular endothelium [3]. The proteinaceous pulmonary edema and intraalveolar hemorrhage, which occurs as result of increased alveolar permeability, may lead to abnormalities in the pulmonary surfactant composition and function [4]. We recently reported that the alteration of the surfactant system in oleic acid-induced ARDS could also be due to the type II cell response to the injury [5–7]. However, the pathogenesis of surfactant alteration in ARDS is poorly understood. Likewise, the mechanism responsible for the injury in the alveolar-capillary membrane remains to be elucidated.

Several authors have reported potential causes of oleic acid-induced ARDS such as activation of blood-cells [8], oxygen radical generation [9] and lung-derived arachidonic acid metabolites [10]. On the other hand, several lines of evidence [11,12] indicated that lysophosphatidylcholine (LPC) increases airway and capillary permeability. The amount of LPC required to produce functional and morphological effects comprises only a few percent of total lung phospholipids [12]. Therefore, minor abnormalities in the formation or removal of this compound might lead to lung membrane disfunction. We recently reported that the content of LPC increased in bronchoalveolar lavage in oleic acid-induced ARDS and that this increase was not correlated with a rise in the phospholipase A activity in both bronchoalveolar lavage and plasma [5]. Little is known about the rate of synthesis and clearance of LPC in lung under these pathological conditions. Accordingly, in the present work we investigated the metabolism of LPC in lung microsomal membranes from rabbits under respiratory distress induced by oleic acid. In addition, the lipid

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composition and fluidity of these membranes were studied as well as their capacity to incorporate [^{14}C]glycerol 3-phosphate into glycerolipids.

Materials and Methods

Materials

The following materials were purchased from the Radiochemical Centre (Amersham, Bucks, U.K.): L-[U-^{14}C]glycerol 3-phosphate, CDP[*Me*-^{14}C]choline, 1,2-di[1-^{14}C]oleoyl-*sn*-glycero-3-phosphocholine, [1-^{14}C]palmitoyl-CoA and 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine. Bovine serum albumin (Fraction V, fatty acid free), coenzyme A, ATP, EDTA, dithioerythritol (DTE), cytidine 5′-diphosphocholine, L-glycerol 3-phosphate, palmitoyl-CoA, palmitic acid, oleic acid, 1-palmitoyl-*sn*-glycero-3-phosphocholine, dioleoyl-*sn*-glycero-3-phosphocholine, L-phosphatidylethanolamine from soybean, 1,2-dioleoyl-*sn*-glycerol and scintillation reagents were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 1,6-Diphenyl-1,3,5-hexatriene (DPH) was a product of Aldrich Chem. Co., Steinheim, F.R.G. All other reagents (analytical grade) were obtained from Merck A.G., Darmstadt, F.R.G.

Methods

Animals. Adult male New Zealand White rabbits, weighing 2.0 kg, were used in the experiments. Animals were free of respiratory disease and housed isolated from all other laboratory animals. They were kept in individual cages under identical conditions of temperature (23 ± 2° C) and humidity (70%) and were fed a standard diet (Pamlanb Lab).

Oleic acid model. Prior to oleic acid administration and during the course of the experiment rabbits were anesthetized with slow intravenous injection of ketamine (10 mg/kg) by intermittent doses via the ear vein. Lung injury was induced by injection of a constant amount of pure oleic acid (100 μl/kg) into the infusion system conected to the ear vein. The oleic acid was allowed to enter into the ear vein with a constant flow of normal saline. The length of tubing that oleic acid had to run over until reaching the ear vein was 25 cm.

Arterial blood gases and pH were continously monitored (Gas Check 938 AVL) in blood from the catheterized femoral artery. During the course of the experiment, sodium, potassium, calcium and glucose were also determined using an Automated Stat Routine Analyzer (Astra-4, Beckman) and creatinine with a Creatinine Analyzer 2 (Beckman). Lungs were removed 150 min after oleic acid administration.

Preparation of lung microsomal membranes. Lung tissue was excised and carefully washed with ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 0.15 M KCl, 1 mM EDTA, 1 mM DTE and 5% glycerol. A 20% homogenate was prepared in the same buffer in a Potter-Elvehjem homogenizer provided with a Teflon pestle. The microsomal fraction was obtained as previously described [13]. Microsomal pellets were resuspended in ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 0.15 M KCl and 1 mM DTE and resedimented at $150\,000 \times g$ for 45 min. All procedures were carried out at 0–4° C. Microsomal pellets were resuspended in the last buffer and immediately used for enzyme assays. Microsomal membranes stored at −80° C were used for lipid analysis and fluorescence polarization studies. Protein concentration was determined by the method of Lowry et al. [14], with bovine serum albumin as standard.

Membrane lipid analysis. Lipids were extracted from the membrane preparations according to Bligh and Dyer [15]. The individual phospholipids were separated by two-dimensional chromatography on pre-coated activated silica-gel type 60 G thin-layer plates using as solvent systems chloroform/methanol/water (75 : 25 : 3, v/v) and chloroform/methanol/acetic acid/water (90 : 40 : 12 : 2, v/v). Phospholipids were quantitated by phosphate analysis as described by Rouser et al. [16]. Neutral lipids were separated using hexane/ether/acetic acid (70 : 30 : 1, v/v) as developing system. Diacylglycerols were quantitated by analysis of their fatty acid methyl esters in a Hewlett-Packard gas chromatograph and columns of 10% Sp-2330 on 100/200 Chromosorb W (Supelco Inc) at 180° C. Known amounts of heptadecanoic acid (17 : 0) were used as internal standard prior to the separation procedures to allow for recovery losses. Fatty acid composition of microsomal membrane phospholipids was also analyzed by gas chromatography of their fatty acid methyl esters. Total membrane cholesterol was determined enzymatically using the Merkotest Cholesterol Kit (Merck A.G.).

Fluorescence polarization analysis. Microsomal membranes (0.1 mg proteins) were incubated for 30 min at 37° C with 13.5 nmol of 1,6-diphenyl-1,3,5-triene (DPH) in a final volume of 1.5 ml with 50 mM Tris-HCl buffer (pH 7.4) containing 0.5 mM DTE and 0.15 M KCl.

Fluorescence polarization measurements were performed at 25° C with a Perkin Elmer MPF 44 E spectrofluorimeter, at an excitation wavelength of 365 nm. Measurement conditions and corrections have been described earlier [17].

Membrane lipid fluidity may be defined as the reciprocal of the lipid order parameter (S_{DPH}). Fluorescence polarization values (P_{DPH}) can be quantitatively converted into S_{DPH} using a semi-empirical relationship [18,19] (r_o, the maximal limiting anisotropy, was taken as 0.4).

Enzyme assays. Microsomal phospholipase A was assayed using as substrate 1.7 mM 1,2[1-^{14}C]dioleoyl-*sn*-glycero-3-phosphocholine (spec. act. 0.02 Ci/mol) and 0.7 mM L-phosphatidylethanolamine (from soybean) in 100 mM Tris-HCl buffer (pH 9.5) containing 7

mM $CaCl_2$ and sonicated at 4° C with ten bursts of 30 s at 150 watts in a MSE sonifer. The incubation mixture contained 1 mM di[^{14}C]oleoylphosphatidylcholine, 0.42 mM phosphatidylethanolamine, 4.2 mM $CaCl_2$ and 0.2 mg of fresh microsomal protein in a final volume of 0.5 ml with 80 mM Tris-HCl buffer (pH 8.5), 0.4 mM DTE and 60 mM KCl. This mixture was sonicated for 5 min at 4° C in a water bath sonicator (Millipore) before the reaction was started by introducing the incubation mixture in a water bath set at 37° C for 60 min. Blank incubations without microsomal membranes were included in each experiment and the results were corrected for the background radioactivity thus extracted.

Acyl-CoA : lysophosphatidylcholine acyltransferase activity was determined as described in Ref. 20 by incubation of 15 nmol of [1-^{14}C]palmitoyl-CoA (spec. act. 1 Ci/mol), 0.1 μmol of 1-palmitoyl-*sn*-glycero-3-phosphocholine and 0.1 mg of microsomal protein in a final volume of 0.5 ml with 90 mM Tris-HCl buffer (pH 7.4), 0.2 mM DTE, 2.4 mM $MgCl_2$ and 30 mM KCl. The incubation was carried out for 30 min at 37° C. Both substrates were previously sonicated at 0–4° C at 120 watts with two bursts of 30 s. The acyl-CoA hydrolase activity was measured simultaneously to the acyltransferase activity.

CoA-independent transacylase activity was assayed as described in Ref. 21 using 1-[1-^{14}C]palmitoyl-*sn*-glycero-3-phosphocholine as acyl acceptor and endogenous phospholipids as acyl donors. The incubation mixture contained 0.1 μmol of the labelled lysophospholipid (spec. act. 0.05 Ci/mol) and 0.3 mg of fresh microsomal protein in a final volume of 1 ml with 85 mM Tris-HCl buffer (pH 7.4) containing 0.3 mM DTE, 2.2 mM $MgCl_2$ and 45 mM KCl. The incubations were carried out for 30 min at 37° C. Microsomal lysophospholipase activity was evaluated simultaneously to microsomal transacylase activity by analysis of [^{14}C]palmitic acid which was separated from the labelled lyso- and phosphatidylcholine by thin-layer chromatography. Conditions for microsomal lysophospholipase assay were similar to those described [22] in rabbit heart.

Incorporation of [^{14}C]glycerol 3-phosphate into phosphatidic acid, diacylglycerols, phosphatidylcholine and triacylglycerols was assayed as previously described [13] with some modifications. In summary, incubations were carried out with fresh microsomes (0.6 mg proteins) for 45 min at 37° C in a reaction mixture containing 4 mM L-[U-^{14}C]glycerol 3-phosphate (spec. act. 0.1 Ci/mol), 0.2 mM palmitate bound to bovine serum albumin (BSA) (1 mg BSA/100 nmol palmitate), 0.1 mM CoA, 1 mM ATP, 0.4mM CDPcholine and 5 mM $MgCl_2$ in a final volume of 1 ml with 50 mM Tris-HCl buffer (pH 7.4), containing 1 mM DTE and 0.15 M KCl. A time course of the experiment was previously done [23] to choose the optimal time for these studies.

CDPcholine : diacylglycerol cholinephosphotransferase was determined by incubation of 0.4 mM CDP[*Me*-^{14}C]choline (spec. act. 0.2 CI/mol), 0.1 mM 1,2-dioleoyl-*sn*-glycerol (prepared by sonication in a solution of 0.1% Tween 20 as in Ref. 24), 10 mM $MgCl_2$, 1.6 mM DTE, 90 mM KCl and 0.3 mg microsomal protein in a final volume of 0.5 ml with 70 mM Tris-HCl buffer (pH 8). The incubation was carried out for 30 min at 37° C.

The incorporation of choline from CDP[*Me*-^{14}C]choline into phosphatidylcholine was also evaluated using 0.2 μmol of the labelled substrate (spec. act. 0.2 Ci/mol) and the diacylglycerol generating system: 2 μmol of L-glycerol 3-phosphate, 0.1 μmol of palmitate bound to BSA, 5 μmol of $MgCl_2$, 0.05 μmol of CoA and 0.5 μmol of ATP. The incubation was started by addition of 0.3 mg of microsomal protein in a final volume of 0.5 ml with 70 mM Tris-HCl buffer (pH 8) containing 1 mM DTE and 0.1 M KCl. This mixture was incubated for 30 min at 37° C.

Reaction product analysis. All assay reactions were stopped by adding 5 volumes of chloroform/methanol (2 : 1, v/v). After total lipid extraction, the lipid classes were fractionated by one-dimensional thin-layer chromatography on silica gel plates. Neutral lipids were separated as described above and phospholipid classes using chloroform/methanol/water (65 : 25 : 4, v/v) as chromatographic solvent. Lipid areas of chromatograms were visually detected by exposure to I_2 vapor and scraped off into counting vials containing 15 ml of scintillation cocktail (toluene scintillation mixture [0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis(5-phenyloxazol-2-yl)benzene in scintillation-grade toluene], Triton X-100 and water (10 : 5 : 1, v/v)). Samples were counted in a Packard 3255 scintillation spectrometer with external-standard correction for quenched samples.

Results

Oleic acid-induced lung injury

Intravenous infusion of pure oleic acid produced in rabbits a syndrome of hemorrhagic pulmonary edema with diminishing arterial pO_2 (Fig. 1) and acidosis (Fig. 2). The lungs were edematous, congested and in some areas with a liver-like appearance.

Composition and structural studies in microsomal membranes

Table I shows the major composition data of lung microsomal membranes isolated from control rabbits and rabbits under oleic acid-induced ARDS (OA-ARDS).

The amount of microsomal protein per g of total lung decreased in OA-ARDS likely due to the interstitial and intraalveolar edema occurring in lungs 150 min after oleic acid administration. On the other hand, the ratios of total phospholipids, cholesterol and di-

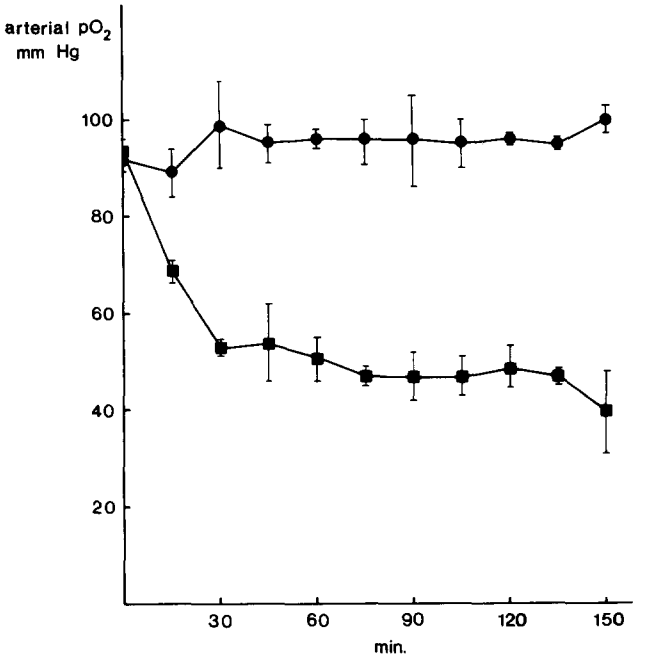

Fig. 1. Arterial pO_2 from control and OA-ARDS groups during the course of the experiment. The data are mean values ± S.D. for four animals. ●, control group; ■, group of animals under oleic acid-induced adult respiratory distress syndrome (OA-ARDS).

acylglycerols to proteins decreased in microsomes from injured lungs.

Table II shows the ratios of individual phospholipids to proteins. The amounts of phosphatidylcholine (PC), phosphatidylethanolamine, and phosphatidylglycerol per mg protein significantly decreased under oleic acid-induced injury. Percentages of each phospholipid class are also listed in Table II. The proportions of LPC and sphingomyelin significantly increased while that of PC decreased in OA-ARDS. Thus, the PC/LPC molar ratio dropped from 112 ± 17 in control group to 53.4 ± 2.4 in OA-ARDS group.

Table III shows the values of P_{DPH} and S_{DPH}, which reflect the packing of apolar moieties in the whole membrane. P_{DPH} and S_{DPH} values remained unchanged in the OA-ARDS group as compared to those

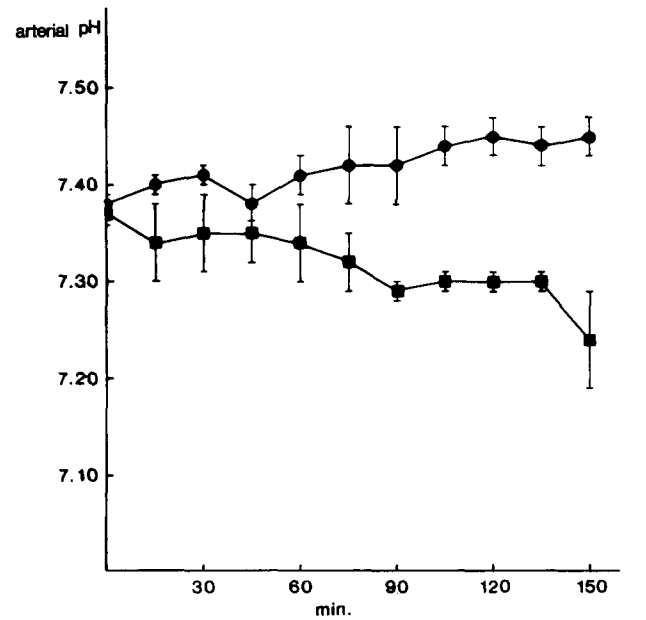

Fig. 2. Arterial pH from control and OA-ARDS groups during the course of the experiment. The data are mean values ± S.D. for four animals. ●, control; ■, OA-ARDS.

TABLE I

Major composition data of lung microsomal membranes

All data are means ± S.D. for four animals. Student's *t*-test was used to analyze the difference between the means, and $P < 0.05$ was considered significant (** $P < 0.01$ and * $P < 0.05$ vs. control). OA-ARDS, oleic acid-induced adult respiratory distress syndrome.

Parameter	Control	OA-ARDS
Protein (mg/g tissue)	10.1 ± 1.6	5.6 ± 0.4 **
Phospholipid (nmol/mg protein)	480.7 ± 90.0	237.0 ± 14.0 **
Cholesterol (nmol/mg protein)	165.8 ± 10.0	118.2 ± 16.7 *
Diacylglycerol (nmol/mg protein)	26.1 ± 2.7	19.9 ± 1.0 *

TABLE II

Major phospholipid classes of lung microsomal membranes

Values are means ± S.D., $n = 4$. Student's *t*-test was used for statistical analysis (** $P < 0.01$ vs. control). LPC, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipids.

PL	Control		AO-ARDS	
	nmol PL/ mg protein	mol%	nmol PL/ mg protein	mol%
LPC	2.7 ± 0.3	0.6 ± 0.1	2.5 ± 0.2	1.0 ± 0.1 **
SM	39.4 ± 10.1	8.3 ± 1.0	28.0 ± 3.8	11.8 ± 0.9 **
PC	306.2 ± 69.0	64.6 ± 1.3	131.0 ± 8.9 **	55.5 ± 6.6 **
PS + PI	49.5 ± 15.0	10.4 ± 2.2	30.4 ± 3.9	12.8 ± 0.9
PE	65.1 ± 4.3	13.8 ± 2.3	40.6 ± 8.4 **	17.0 ± 2.5
PG	10.2 ± 1.7	2.2 ± 0.6	4.4 ± 1.0 **	1.9 ± 0.5

TABLE III

Lipid fluidity of lung microsomal membranes

Lipid structural order parameter (S_{DPH}) was calculated from fluorescence polarization values (P_{DPH}) as reported under Methods. The degree of unsaturation of phospholipid acyl chains was calculated as [% monoenes + 2(% dienes) + 4(% tetraenes)]/100 from fatty acid composition values. All data are means ± S.D. for four animals.

Parameter	Control	OA-ARDS
P_{DPH} (25 °C)	0.312 ± 0.003	0.322 ± 0.009
S_{DPH} (25 °C)	0.724 ± 0.004	0.743 ± 0.020
Unsaturation degree	0.780 ± 0.020	0.830 ± 0.030
Cholesterol/phospholipid (molar ratio)	0.360 ± 0.070	0.490 ± 0.070

TABLE IV

Fatty acid analysis of lung microsomal phospholipids

Values are means ± S.D., $n = 4$. FA, fatty acids; PL, phospholipids.

Fatty acid	Control (μg FA/mg PL)	OA-ARDS (μg FA/mg PL)
16:0	342.8 ± 23	276.5 ± 25
16:1	16.4 ± 3	19.4 ± 5
18:0	66.9 ± 18	91.1 ± 19
18:1	125.2 ± 13	143.6 ± 14
18:2	107.9 ± 17	91.1 ± 18
20:4	48.9 ± 7	56.5 ± 6

of the control group. Therefore, the fluidity of microsomal membrane core did not alter in oleic acid-induced ARDS. Likewise, the cholesterol/phospholipid molar ratio and the degree of unsaturation of phospholipid acyl chains, which are the major parameters related to membrane lipid fluidity, did not significantly change. The analysis of fatty acids of microsomal phospholipids (Table IV) shows minor changes in the content of palmitic, oleic and arachidonic acids.

Metabolic studies

In view of the marked decrease of PC/LPC molar ratio in injured lung microsomes, the microsomal enzyme activities involved in the formation and clearance of LPC were studied (Table V).

The specific activity of microsomal phospholipase A exhibited a significant increase in pathological rabbits. Earlier studies [25] have shown that the phospholipase A activity of rabbit lung microsomes is mainly of the A_2-type. Hence it is likely that the phospholipase A activity measured in the present study using 1,2-di[1-^{14}C]oleoyl-PC as substrate, represents predominantly phospholipase A_2 activity. Many phosphalipases A_2 hydrolyze exogenous phosphatidylethanolamine (PE) more efficiently than PC, when assayed without detergents [25,26]. However, this does not reflect enzyme specificity but rather a better association of PE with biomembranes due to its physicochemical properties [26]. In the present study we used a mixed system consisting of 70 mol% di[^{14}C]oleoyl-PC and 30 mol% L-PE from soybean in the incubation mixture in order to increase the association of PC with the enzyme-containing membranes in the absence of detergents.

Concerning the enzyme activities involved in the removal of LPC, the microsomal lysophospholipase activity did not change in lung microsomes from rabbits under oleic acid injury whereas acyl-CoA : lysophosphatidylcholine acyltransferase activity decreased (Table V). This decrease was not caused by an increase of the acyl-CoA hydrolase activity which was measured simultaneously to the acyltransferase activity since both compete for the same substrate [20]. Values of specific activity of acyl-CoA hydrolase (1.1 ± 0.05 nmol FA/min per mg prot) were similar in control and pathological groups.

On the other hand, the acylation of LPC through CoA-independent transacylation increased 2-fold in injured lung microsomes (Table V). This enzyme activity was assayed using 1-[1-^{14}C]palmitoyl-LPC as acyl acceptor and endogenous phospholipids as acyl donors. In order to avoid the possible contamination with the lysophosphatidylcholine: lysophosphatidylcholine acyltransferase activity from the soluble fraction [27], microsomal pellet was washed and resedimented as described above.

The incorporation of ^{14}C-glycerol 3-phosphate into microsomal glycerolipids is shown in Table VI. In the distressed animals the incorporation of glycerol 3-phosphate into phosphatidylcholine, triacylglycerols and, their common precursors, phosphatidic acid and diacylglycerols was decreased. The marked decrease in the incorporation of glycerol 3-phosphate into PC and TG

TABLE V

Specific activities of lung microsomal enzymes involved in lysophosphatidylcholine metabolism

The data are mean values ± S.D. for four animals. Triplicate or duplicate determinations were assayed from each of the animals. Experimental details are given in the text. Student's *t*-test was used for statistical analysis (* $P < 0.05$ vs. control).

Enzyme activity	Control	OA-ARDS
Phospholipase A (nmol LPC/h per mg protein)	33.7 ± 6.3	48.8 ± 4.8 *
Lysophospholipase (nmol FA/min per mg protein)	8.8 ± 0.1	7.7 ± 0.9
Acyl-CoA : LPC acyltransferase (nmol PC/min per mg protein)	1.2 ± 0.1	0.8 ± 0.2 *
CoA-independent transacylase (nmol PC/min per mg protein)	0.5 ± 0.2	1.1 ± 0.2 *

TABLE VI

Incorporation of L-[U-^{14}C]glycerol 3-phosphate into glycerolipids by lung microsomes

Values are means ± S.D. for the number of animals indicated in parenthesis. Duplicate determinations were assayed from each of the animals. Experimental details are given in the text. Student's *t*-test was used for statistical analysis (** $P < 0.01$ and * $P < 0.05$ vs. control). PA, phosphatidic acid, DG; diacylglycerol, PC; phosphatidylcholine; TG, triacylglycerol.

Lipid class	[^{14}C]Glycerol-3-*P* incorporated	
	control ($n = 3$)	OA-ARDS ($n = 4$)
PA	17.7 ± 1.7	13.1 ± 2.0 *
DG	9.3 ± 1.9	5.5 ± 1.1 *
PC	10.5 ± 1.8	3.3 ± 0.7 **
TG	5.1 ± 0.9	3.2 ± 0.6 *

TABLE VII

Incorporation of choline from CDP[Me-^{14}C]choline into phosphatidylcholine by lung microsomes

Values are means ± S.D., $n = 4$. Duplicate determinations were assayed from each of the animals. (a) Exogenous diacylglycerol: 1,2-dioleoyl-L-*sn*-glycerol in 100 mM Tris-HCl buffer (pH 8.5) containing 0.1% Tween 20 (final concentration 0.1 mM); (b) Diacylglycerol generating system: 4 mM L-α-glycerophosphate, 0.2 mM palmitate bound to BSA, 5 mM $MgCl_2$, 0.1 mM CoA and 1 mM ATP. Experimental details are given in the text. Student's *t*-test was used for statistical analysis (* $P > 0.05$ vs. control).

Group	nmol [^{14}C]choline incorporated	
	exogenous DG (a) (nmol PC/mg protein)	endogenous DG (b) (nmol PC/mg protein)
Control	1.8 ± 0.1	2.0 ± 0.3
OA-ARDS	1.4 ± 0.2 *	0.9 ± 0.4 *

could be a consequence of the decreased incorporation of this precursor into DG. However, the possibility that the enzyme cholinephosphotransferase could also be altered in injured lung microsomes can not be excluded since this enzyme has been shown to require PC for maximum activity [28] and is inhibited by enhanced content of LPC [29]. Accordingly, experiments were performed to evaluate the incorporation of choline from CDP[*Me*-^{14}C]choline into PC by using either exogenous DG or a DG generating system (Table VII).

The inhibition percentage of PC synthesis using CDP[^{14}C]choline and the de novo generated DG (55.5%) (Table VII) was similar to that found using glycerol 3-phosphate (58%) as labelled substrate (Table VI). On the other hand, a 20% decrease of PC synthesis from CDP[^{14}C]choline and exogenous DG was found (Table VII) suggesting that the decreased incorporation of glycerol 3-phosphate into PC could be due not only to the reduction of the de novo synthesis of DG but also to the partial inhibition of cholinephosphotransferase activity which might be affected by the altered lipid composition of the microsomal membrane.

Discussion

Intravenous infusion of pure oleic acid in rabbits causes the formation of a severe pulmonary injury which is accompanied by alterations in the microsomal lipid composition and in some membrane-bound enzyme activities involved in glycerolipid metabolism.

There are several aspects of the present results that should prove to be of particular interest.

The first is the alteration of microsomal lipid composition in response to the injury. Particularly remarkable is the decrease of PC content and the increase of LPC content (Table II). The increase of LPC could be related with the enlarged content of this phospholipid observed in bronchoalveolar lavage of pathological rabbits [5]. It is well known that LPC possesses cytolitic and membrane-perturbing properties [30]. Relatively small amounts of this phospholipid impair the barrier functions of the alveolar epithelium, in which the type I pneumocytes are the cells most susceptible to LPC exposure [12].

A key question is what the relative contribution is of both phospholipase A activity and the enzymes involved in LPC removal to the increase of microsomal LPC content in injured lungs. Specific activity of microsomal phospholipase A increased under oleic acid-induced ARDS (Table V). This activity has been reported to increase in lung experimental models which lead to the formation of a lung syndrome such as in septic shock [31] and pneumonitis [32]. Likewise, a rise of phospholipase A_2 activity has been shown to occur in other systems after insult by free radicals [33], trichloroethylene [34] or experimental endotoxic shock [35]. These findings suggest the possible importance of the increase of phospholipase A activity under pathological conditions.

Lysophospholipids generated within the microsomal membrane by action of phospholipase A can either return to the membrane phospholipids via the reacylation pathway or become further degraded by lysophospholipases. These pathways are important mechanisms for regulating the levels of lysophospholipids [36].

The reacylation of LPC by acyl-CoA : lysophosphatidylcholine acyltransferase might be important in the lungs' response to injury since: (a) an increase of oxygen radical production might occur under oleic acid-induced ARDS [9] and hypoxic conditions [37]. In this case, deacylation-reacylation mechanism could be important to replace oxidized fatty acids and restore the fatty acid composition of PC [38]. (b) In type II cells, the reacylation mechanism plays an important role in maintaining a high level of dipalmitoyl-PC, the major active component of pulmonary surfactant [39]. The results reported here reveal that acyl-CoA : lysophosphatidylcholine acyltransferase activity decreases in lung microsomes from rabbits under oleic acid-induced pulmonary injury (Table V). Changes in this enzyme activity during acute alveolar injury has been suggested to be mainly due to the destruction and regeneration of type II cells [40]. These cells are highly enriched with acyl-CoA : lysophosphatidylcholine acyltransferase as compared to whole lung [41].

Alternative mechanisms for lysophospholipid acylation in microsomal membranes, not involving CoA and ATP, have been reported [21,42]. Such direct transfer of acyl groups between intact phospholipids, independently of any known cofactor, has also been studied in this work (Table V). We show that the acylation of 1-palmitoyl-LPC using endogenous lipids as acyl-donors, in the absence of CoA and ATP, increased 2-fold in pathological lung microsomes. Although such

transacylation mechanism may be important in the remodelling of phospholipid molecular species [42], there is however little evidence for a role of this mechanism in regulating LPC levels.

Another alternative for removal of LPC would be its degradation by microsomal lysophospholipase. However this enzyme did not change in pathological lungs. Therefore, injured lung did not increase the clearance of lysophosphatidylcholine via hydrolysis.

Other aspect to the obtained data that should be pointed out concerns the diminished ability of injured lung microsomes to incorporate [^{14}C]glycerol 3-phosphate into phosphatidic acid and diacylglycerol (Table VI). The reduction of the de novo synthesis of diacylglycerol could be related with the lowered level of this compound in injured lung microsomes (Table I). In addition, the decreased incorporation of [^{14}C]glycerol 3-phosphate into PC (Table VI) should be a consequence of the diminished supply of the de novo synthesized diacylglycerol. However, it should be denoted that cholinephosphotransferase activity was also impaired even if exogenous diacylglycerol was used as substrate (Table VII) which agrees with a report of Wichert et al. [43]. It would be of interest to investigate in future studies whether the conversion of choline into PC is affected in the injured lung. Inhibition of phosphocholine cytidylyltransferase, the rate-limiting enzyme in this pathway, may limit the supply of CDPcholine, the second substrate for the cholinephosphotransferase reaction.

The impairment of the microsomal system of glycerolipid metabolism might be due to alterations in the lipid microenvironment in these membranes. Assuming that binding affinities of LPC to some integral membrane proteins are higher than those of other lipids [44], boundary lipids around integral proteins might be displaced, which might in turn interfere with protein structure and enzymic activities.

On the other hand, changes in the activity of some membrane-bound enzymes can be due to changes in membrane lipid fluidity [45]. Therefore, lipid fluidity was measured using fluorescence polarization with diphenylhexatriene as fluorophore which monitors the dynamic behaviour of the hydrophobic regions of the membrane. It is interesting to note that in spite of lipid modifications occurring in these membranes under oleic acid-induced ARDS, no significant change in lipid fluidity parameters was found (Table III).

It is generally assumed that above the thermotropic transition temperature the membrane fluidity varies inversely with the cholesterol/phospholipid molar ratio [21] and directly with the unsaturation degree of phospholipid acyl chains [45]. These factors on which the fluidity depends, remained unchanged in lung microsomal membranes under these pathological conditions (Table III).

In summary, we have found alterations in lipid composition but not in lipid fluidity in lung microsomal membranes from rabbits under oleic acid-induced ARDS. Microsomal enzyme activities involved in LPC metabolism were affected resulting in an increased content of this lysophospholipid in microsomal membranes. In addition, some microsomal enzyme activities involved in glycerolipid metabolism were decreased. Since phospholipids turn over continuously in membranes [36] and the endoplasmic reticulum is the location of the majority of the lipid synthesis [46], the changes found in microsomal membranes could affect this turnover which is involved in the repair and maintenance of membranes. Therefore, lipid alterations in microsomal membranes might be of importance in the pathogenesis of oleic acid-induced ARDS.

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