

Platelet-activating Factor Cytosolic Phospholipase A₂

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Cytosolic Phospholipase A₂ Activity in Neutrophilic Oxidative Stress of Platelet-activating Factor-induced Acute Lung Injury

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Background: The present investigation was performed in rats and isolated human neutrophils in order to confirm the presumptive role of the positive feedback loop of cytosolic phospholipase A₂ (cPLA₂) activation by platelet-activating factor (PAF).

Methods: The possible formation of the positive feedback loop of the cPLA₂ activation and neutrophilic respiratory burst was investigated *in vivo* and *in vitro* by measurement of the parameters denoting acute lung injury. In addition, morphological examinations and electron microscopic cytochemistry were performed for the detection of free radicals in the lung.

Results: Five hours after intratracheal instillation of PAF (5 µg/rat), the lung leak index, lung myeloperoxidase (MPO) activity, the number of neutrophils and the concentration of cytokine-induced neutrophil chemoattractant (CINC) in bronchoalveolar lavage fluid were increased by PAF as compared with those of control rats. The NBT assay and cytochrome-c reduction assay revealed an increased neutrophilic respiratory burst in isolated human neutrophils following exposure to PAF. Lung and neutrophilic cPLA₂ activity were increased following PAF exposure and exposure to hydrogen peroxide increased cPLA₂ activity in the lung. Histologically, inflammatory findings of the lung were observed after PAF treatment. Remarkably, as determined by CeCl₃ cytochemical electron microscopy, increased production of hydrogen peroxide was identified in the lung after PAF treatment.

Conclusion: PAF mediates acute oxidative lung injury by the activation of cPLA₂, which may provoke the generation of free radicals in neutrophils. (*Tuberc Respir Dis* 2007;63:497-506)

Key Words: Acute lung injury, PAF, cPLA₂, Neutrophils

서 론

Phospholipase A₂ (PLA₂)
(pathogenesis) . PLA₂
80,000 dalton cyto-
solic PLA₂ (cPLA₂)
cytokine, PLA₂,
(C5a)
cytokine
(adhesion molecule)

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NADPH oxi-
dase O₂^{•-} (superoxide anion)
NADPH oxidase Dana⁷
PLA₂
PLA₂
(chemotaxis)
platelet-activating factor (PAF)
NADPH oxidase
(adhesion)⁸
PAF가
,
,
,
9. PAF
,
가
10,11
PAF
cPLA₂
PAF가 cPLA₂
PAF
가

대상 및 방법

1. 시약 및 실험동물

® (enflurane), cPLA₂
palmitoyl-2(9,10(N)-³H palmi-
toyl)-phosphatidylcholine (³H-DPPC) NEN life science
products (Boston, MA, USA), ¹²⁵I-labelled
bovine serum albumin ICN radiochemical (Irvine, CA,
USA) Sigma chem-
ical company (St. Louis, MO, USA)
300g Sprague-Dawley

non-paired sampling

2. 흰쥐에서의 급성 폐손상의 유도

PAF
5 µg PAF 0.5 ml 0.25%
bovine serum albumin¹²
0.5 ml 4.5 mM
PAF 3.0 ml

0.5 ml
PAF,
가
3. 단백누출지수(lung leak index)의 측정
PAF 4 30
1.0 µCi ¹²⁵I-labelled bovine
serum albumin 30 Harvard rodent
ventilator (U.K)
1.0 ml
Masterflex perfusion pump (Cole Parmer,
USA)
(lung
leak index, LLI) / 1.0 ml

4. 폐장의 myeloperoxidase (MPO) 활성도의 측정

PAF 5
Goldblum¹³ MPO

5. 폐포세척액 내의 호중구의 산정

PAF 5
8.0 ml (bron-
choalveolar lavage, BAL) BAL
1,500 rpm
0.1 ml 가
70~100 µl
Wright

6. 폐포세척액 내의 cytokine-induced neutrophil chemoattractant (CINC) 농도의 측정

CINC Wittwer¹⁴
Sandwich ELISA

7. NBT검사를 이용한 폐포세척액 내의 호중구의 respiratory burst의 확인

PAF 5 respi-

(Table 1).

2. Lung MPO activity

	MPO	
(U/g of wet lung)	2.60±0.39	PAF
	30.07±2.57	가
(p<0.001).	PAF가	
	(Table 1).	

Table 1. Comparisons of lung leak index, lung MPO, BAL PMNLs, CINC concentration between sham and PAF-treated rats

	Sham	PAF-treated
LLI	0.080±0.004 (n=8)	0.177±0.018* (n=12)
MPO (U/g of lung)	2.60±0.39 (n=11)	30.07±2.57* (n=9)
BAL PMNS (millions/two lungs)	0.313±0.238 (n=8)	3.34±0.419* (n=8)
CINC (pg/ml of BALF)	39.3±15.2 (n=6)	455.5±56.7* (n=13)

Values are given as mean±S.E.

n indicates number of experiments.

MPO: myeloperoxidase; BAL PMNL: bronchoalveolar lavage polymorphonuclear leukocyte; CINC: cytokine induced neutrophil chemoattractant; PAF: platelet-activating factor; LLI: lung leak index.

*p<0.001, sham vs. PAF.

Table 2. Comparison of indices of neutrophilic oxidative stress between sham and PAF-treated neutrophils

	Sham	PAF-treated
NBT (%)	3.5±1.1 (n=8)	39.8±5.3* (n=6)
Cyto-c reduction (nmol/2×10 ⁶ cells)	2.7±1.1 (n=8)	18.2±2.6* (n=13)

Values are given as mean±S.E.

n indicates number of experiments.

NBT assay was carried out with collected neutrophil from BALF of rats and cytochrome-c reduction assay was performed with isolated human neutrophils.

PAF: platelet-activating factor.

*p<0.001, sham vs. PAF.

3. Enumeration of BAL neutrophils

	(millions/two lungs)	PAF
	0.313±0.238	가
	3.34±0.419	(p<0.001)
(Table 1).		

4. BAL CINC concentration

	CINC	(pg/ml of BALF)
	39.3±15.2	PAF
56.7	가	455.5±
		(p<0.001)(Table 1).

5. NBT 검사

NBT	formazan (%)
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Table 3. Stimulating effects of PAF on the cPLA₂ activity in the lung and isolated human neutrophils

	Sham	PAF-treated
Lung cPLA ₂ (mU/g of lung)	6.58±0.24 (n=13)	10.95±0.82* (n=17)
PMNL cPLA ₂ (mU/2×10 ⁶ cell)	12.64±0.87 (n=9)	15.71±0.47* (n=15)

Values are given as mean±S.E.

n indicates number of experiments.

PAF: platelet-activating factor; cPLA₂: cytosolic phospholipase A₂.

*p<0.001, sham vs. PAF.

Table 4. Effect of hydrogen peroxide on the lung cPLA₂ activity

	Sham	H ₂ O ₂ -treated
	(n=6)	(n=8)
cPLA ₂ activity (mU/g of lung)	7.88±0.70	20.93±1.20*

Values are given as mean±S.E.

n indicates number of experiments.

PAF: platelet-activating factor; cPLA₂: cytosolic phospholipase A₂.

*p<0.001, sham vs. PAF.

3.5±1.1 PAF 39.8±5.3
가 (p<0.001) PAF
respiratory burst
가 (Table 2).

6. Cytochrome-c 환원검사

PAF가

cytochrome-c

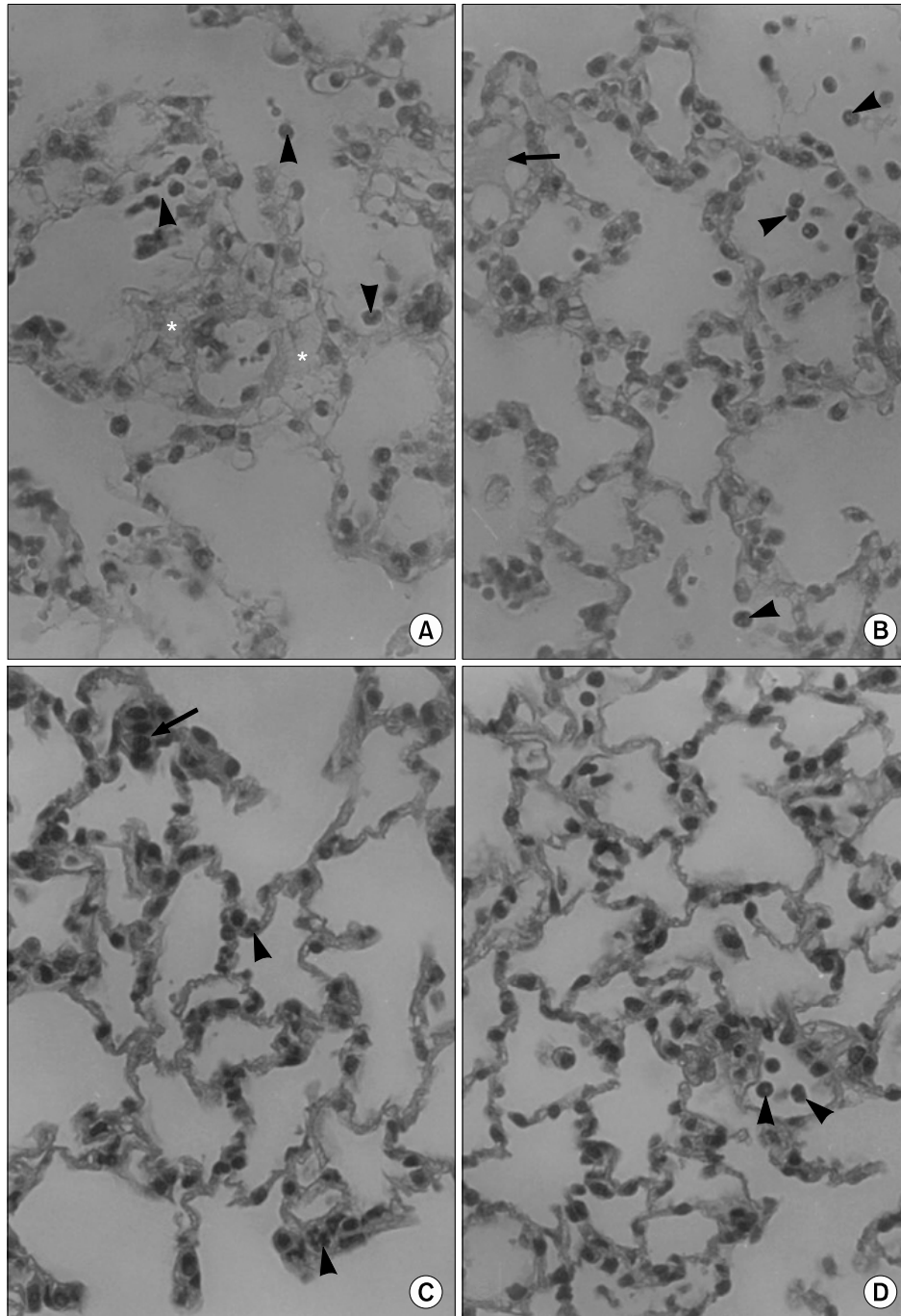


Figure 1. Histological changes in the lung after instillation of PAF (5 μ g) into the trachea. Perivascular cuffing (asterik, A), interstitial edema and infiltration of inflammatory cells were found (A, B). Accumulation of inflammatory cells in vascular lumen, interstitium and alveoli were found (arrow head, C, D). $\times 200$.

Table 2
(cytochrome c-reduced, nmol/2×10⁶ cells) PAF 2.72±1.17
18.24±2.62 가 (p<0.001). 12.64±0.87 가 (p<0.001)(Table 3).

7. 폐장 및 호중구에서의 cPLA₂ 활성도

PAF PLA₂ (U/g of wet lung) 7.88±0.70
6.58±0.24 10.95±0.82 가 (p<0.001)(Table 4).
가 (p<0.001)(Table 3).
PAF cPLA₂ (U/2×10⁶ cells) 20.93±1.20

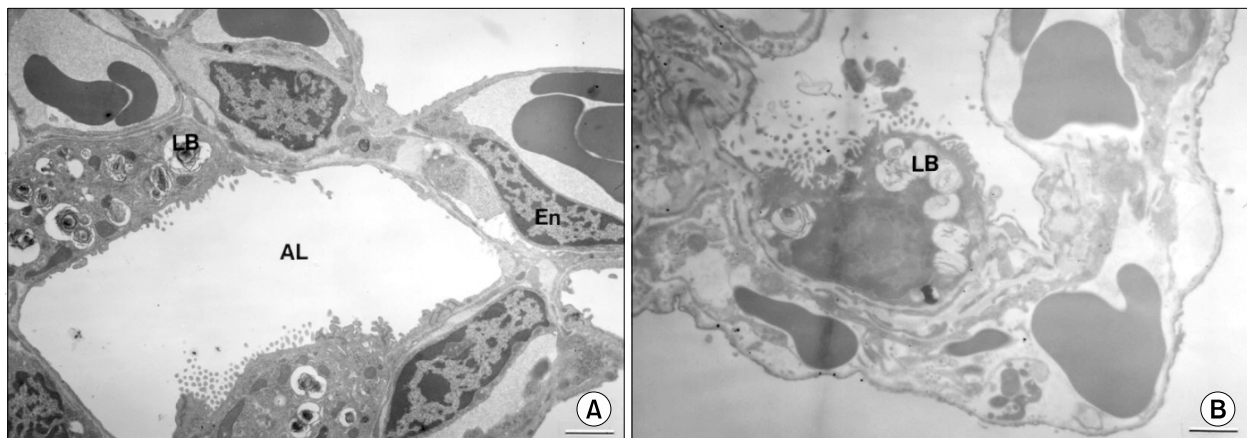


Figure 2. Electron microscopic finding of normal lung (A). Lamellar bodies, endothelial cells and type I alveolar cells were well preserved. In CeCl₃ electron microscopic cytochemistry (B), deposits of cerrous perhydroxide were not found indicating oxidants were not generated in the normal lung. Bar indicates 2.5 μm. LB: lamellar body; En: endothelial cell; AL: alveolar lumen.

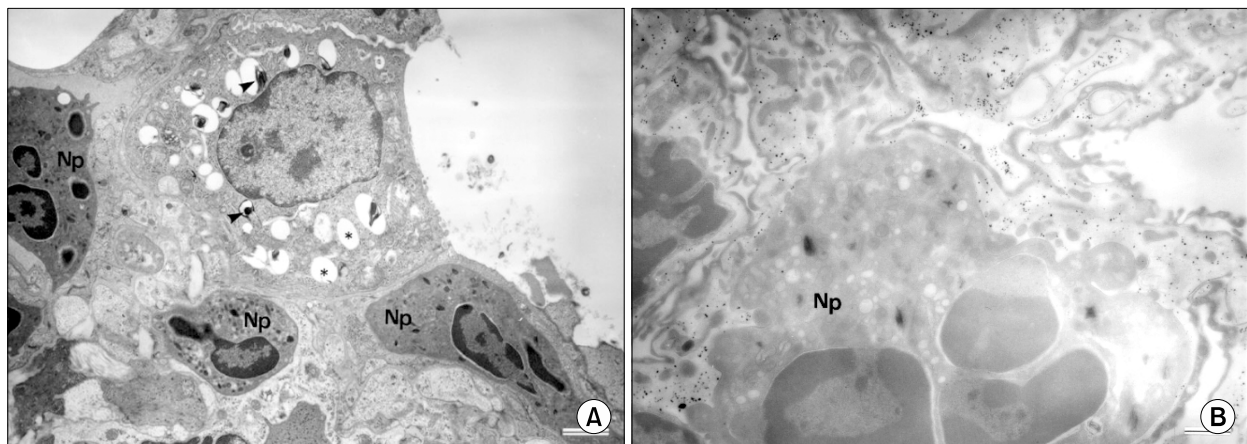


Figure 3. Ultrastructural changes in the lung of the rat given PAF (5 μg) intratracheally. In routine electron microscopic finding (A), the infiltration of neutrophils in interstitium (NP), degeneration of lamellar bodies (arrow head), vacuolization of lamellar bodies (asterik) were noted. NP: neutrophil. Bar indicates 2.0 μm. In CeCl₃ cytochemical electron microscopy, deposits of cerrous perhydroxide granules were indentified in the interstitium and to the proximity of neutrophils (B). NP: neutrophil; Bar indicates 1.0 μm.

8. 세포화학 및 형태학적인 검사

Repine²⁵ 가

PAF 가 . PAF가

(chemotaxis)

(perivascular cuff- NADPH oxidase PAF가

ing) (Figure 1A).

(Figure 1B, C, D).

1, 2 PAF CINC 가 가 . CINC

가 (lamellar body)가 mokine interleukin-8²⁶ . CINC

2 (Figure 2A).

CeCl₃ cytochemical electron mi- PAF PAF가 CINC 가²⁷ .

croscopy cerrous perhydroxide Tamm²⁸ PAF CINC

(Figure 2B).

PAF PAF CINC 가 PAF

2 (Figure 3A).

cerrous perhydroxide 가 CINC 가 PAF

(Figure 3B).

고 찰

(pathogenesis)

PAF CINC 가²⁹ PAF 가

PAF acetylhydrolase PAF 가²⁰ .

PAF 가²¹ acid aspiration PAF CINC

PAF가 respiratory burst NBT PAF

NBT for-

mazan 1, 2

30,31

Martensson³² 2

가

가

가 PAF¹² . Camussi

PAF가

NADPH oxidase²⁴ .

가

cerium chloride
PAF가

가

가 PAF PLA₂ PLA₂ PLA₂

가 PAF

NADPH oxidase³³ 가

가 NADPH oxidase³⁴ Balsinde

PAF가 PLA₂ 가

post-translation translation PAF가³⁵ PAF가 , , cyto-
Nakashima PLA₂ kine,
PAF가 PAF가 cPLA₂

PLA₂ cPLA₂ cPLA₂

PAF 가 가

가 PAF cPLA₂ 가 가

가 Sun³⁶

PAF TNF- α 요 약

PLA₂ 가 가 PAF

PLA₂ 가 가

PAF cPLA₂ 가 가

가

cPLA₂ 가 가

cPLA₂ 가 가

cPLA₂ Boyer cPLA₂

³⁷, Chakraborti Michael³⁸ 가

PLA₂ PAF cPLA₂

방 법: 5 μ g

PAF 0.5 ml 0.25% bovine serum albumin

0.5 ml 4.5 mM

PAF 0.5 ml . 5

MPO , CINC , NBT cyto-
chrome-c cPLA₂

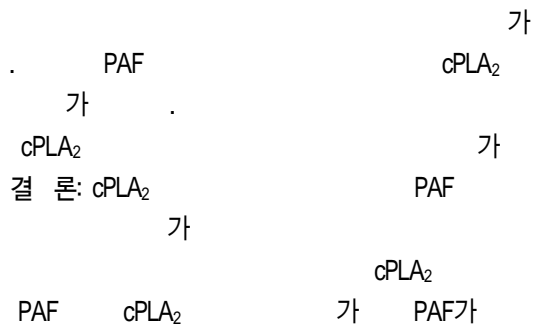
결 과: PAF , MPO, BAL

CINC 가

가 NBT cytochrome-c PAF

respiratory burst 가 ,

PLA₂



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