

Global Phospholipidomics Analysis Reveals Selective Pulmonary Peroxidation Profiles Upon Inhalation of Single-Walled Carbon Nanotubes

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SUPPLEMENTAL MATERIALS

Table S1. *Accumulation of phospholipid hydroperoxides in the lung of mice exposed to SWCNT (pmol/nmol of non-oxidized phospholipid).*

Phospholipids	1day	7 days	28 days
Cardiolipin	17.9 ± 6.4*	42.6 ± 14.5*	N.D.
Phosphatidylserine	3.5 ± 2.9	8.4 ± 3.2*	8.5 ± 2.1*
Phosphatidylinositol	7.5 ± 4.7	12.1 ± 5.5*	1.7 ± 2.2
Phosphatidylethanolamine	1.9 ± 2.9	3.4 ± 2.9	10.5 ± 8.4
Phosphatidylcholine	N.D.	N.D.	1.2 ± 2.1

Lipids were separated by 2D-HPTLC. PL-OOHs were detected using Amplex Red protocol. Data are mean ± SD, n=3-4, *p<0.05 vs. respective control (non-treated mice). N.D. – not detectable. The amounts of PL-OOH in the control samples were in the ranges 18.8 - 32.5, 2.4 - 8.5, 6.7 - 8.5, 6.0 - 8.8 and 2.1 - 5.8 pmol/nmol of non-oxidized phospholipid for CL, PS, PI, PE and PC, respectively. CL – cardiolipin, PE – phosphatidylethanolamine, PC – phosphatidylcholine, PI – phosphatidylinositol, PS – phosphatidylserine.

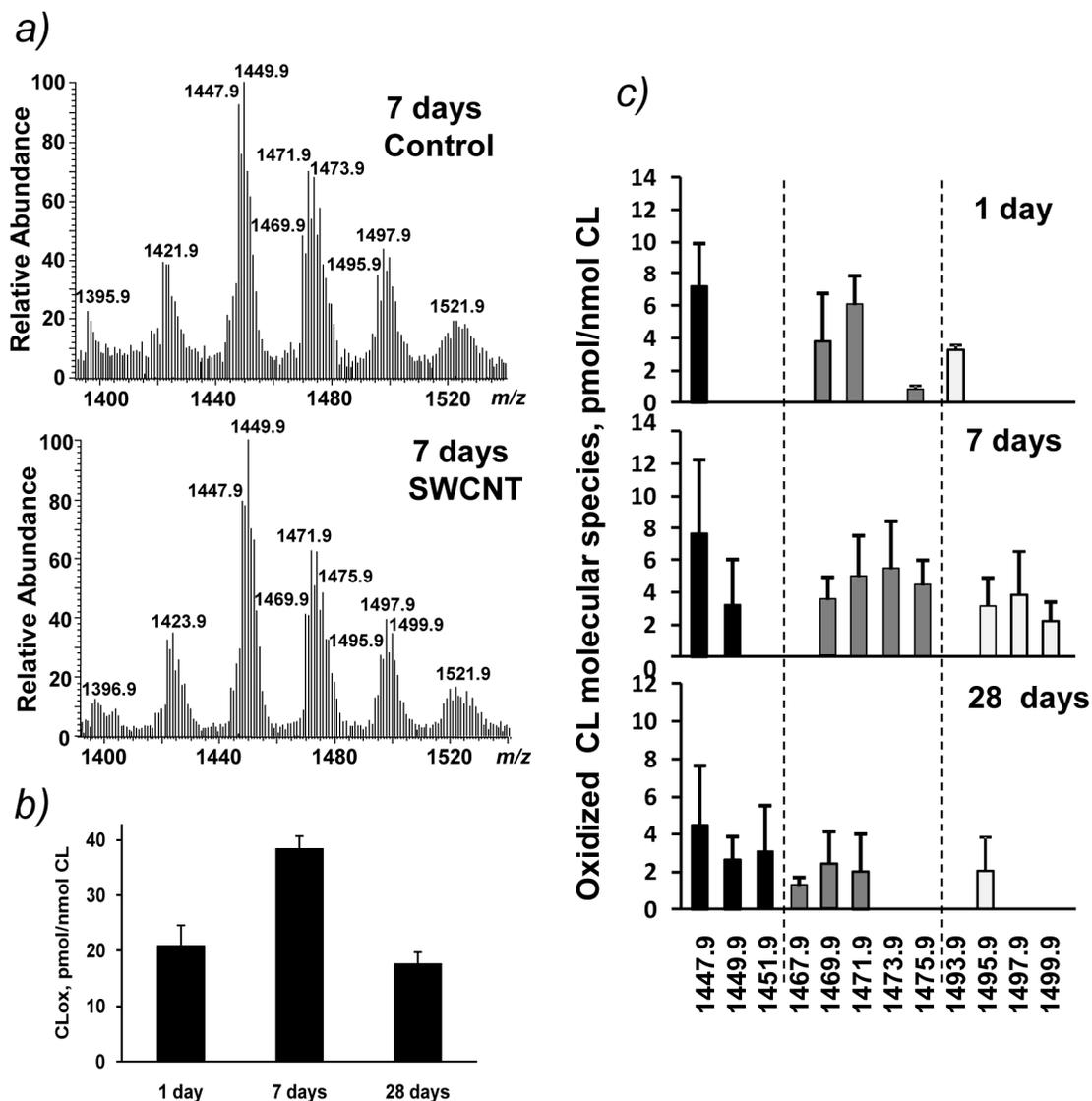


Figure S1. *Assessment of CL molecular species in mouse lungs by LC/ESI-M.*

a). Typical ESI-MS spectra of CL isolated from the lung of control mice and mice exposed to SWCNT. LC/MS analysis revealed a significant oxidation of CL containing mainly C_{18:2} (*m/z* 1447.9, C_{18:2}/C_{18:2}/C_{18:2}/C_{18:2}; 1449.9, C_{18:2}/C_{18:2}/C_{18:2}/C_{18:1}), C_{18:2} and arachidonic (C_{20:4}) (*m/z* 1469.9, C_{18:2}/C_{18:2}/C_{18:3}/C_{20:4}; 1471.9, C_{18:2}/C_{18:2}/C_{18:2}/C_{20:4}; 1473.9, C_{18:1}/C_{18:2}/C_{18:2}/C_{20:4}; 1475.9, C_{18:1}/C_{18:1}/C_{18:2}/C_{20:4}) and C_{18:2} and docosahexaenoic (C_{22:6}) or docosapentaenoic (C_{22:5}) acids (*m/z* 1493.9, 1495.9, C_{18:2}/C_{18:2}/C_{20:4}/C_{20:4}; 1497.9, C_{18:1}/C_{18:2}/C_{20:4}/C_{20:4}; C_{18:1}/C_{18:2}/C_{18:2}/C_{22:6}; 1499.9, C_{18:1}/C_{20:4}/C_{20:4}/C_{18:1}; C_{18:0}/C_{18:2}/C_{18:2}/C_{22:6}; C_{18:0}/C_{18:2}/C_{18:2}/C_{22:5}; C_{18:1}/C_{18:2}/C_{18:2}/C_{22:5}).

b). Effect of SWCNT exposure on oxidation of CL in mouse lung. Quantitative assessment of oxidized CL was performed by LC/MS.

c). Molecular species of CL undergoing oxidative modification in lung after exposure to SWCNT.

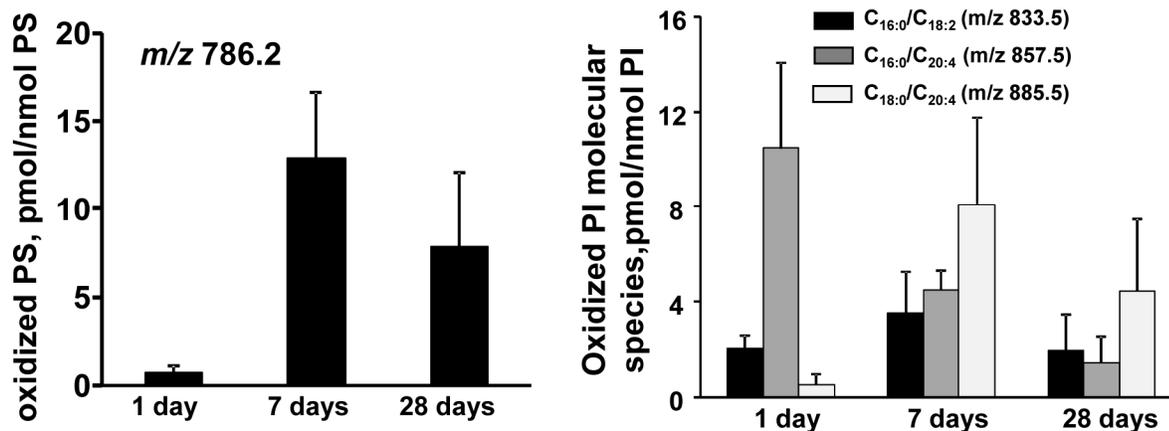


Figure S2. LC/ESI-MS analysis of PS (a) and PI (b) from mice exposed to SWCNT.

The decrease in intensity of molecular ion with m/z 786.2 ($C_{18:0}/C_{18:2}$) and significant accumulation of oxidized PS were detected on days 7 and 28 post-exposure. Three molecular species of PI were underwent oxidative modification induced by SWCNT however the pattern of their oxidation was different. While, PI ($C_{16:0}/C_{20:4}$) was predominantly oxidized on day 1 post-exposure, oxidation of both PI ($C_{18:0}/C_{20:4}$) and ($C_{16:0}/C_{18:2}$) was significantly less pronounced.

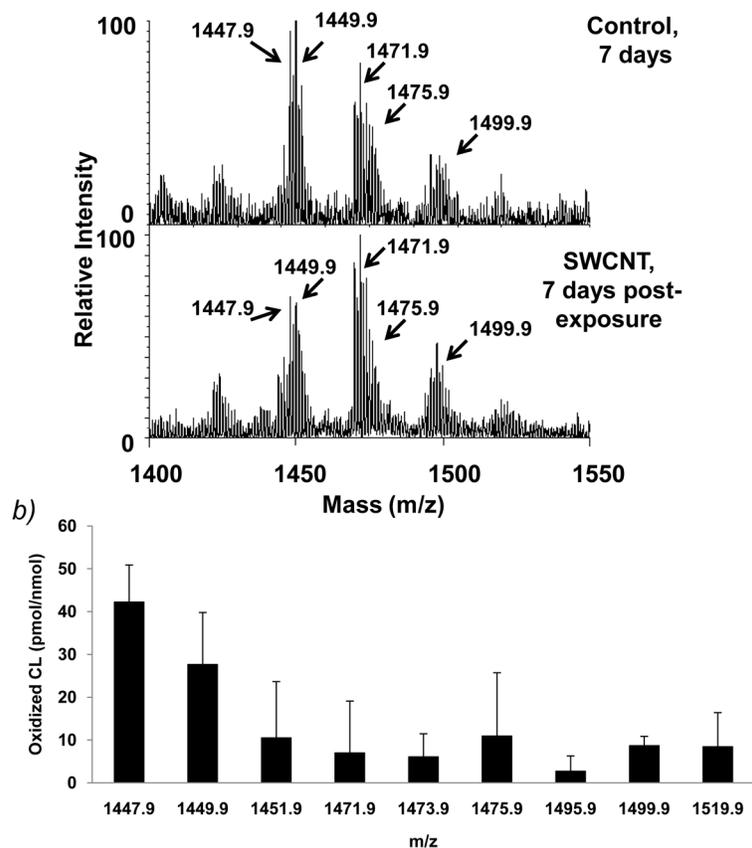


Figure S3. *Assessment of CL molecular species in mouse lung by MALDI-TOF-MS.*

a). Typical MS spectra of CL isolated from control lung and lung of mice exposed to SWCNT (7 day post-exposure). The five molecular clusters containing molecular ions with m/z 1421.9, 1447.9, 1473.9, 1497.9 and 1521.9 were detected. Comparison of day 7 SWCNT samples with controls showed strong losses of species, approximately in proportion to their content of linoleic acid (C18:2), The strongest losses occurred in the tetra- and trilinoleic species (m/z 1447.9 and 1449.9, respectively). Smaller losses were detected among the various di-, and monolinoleic species.

b). Quantitative assessment of oxidized CL molecular species in lung of mice exposed to SWCNT.

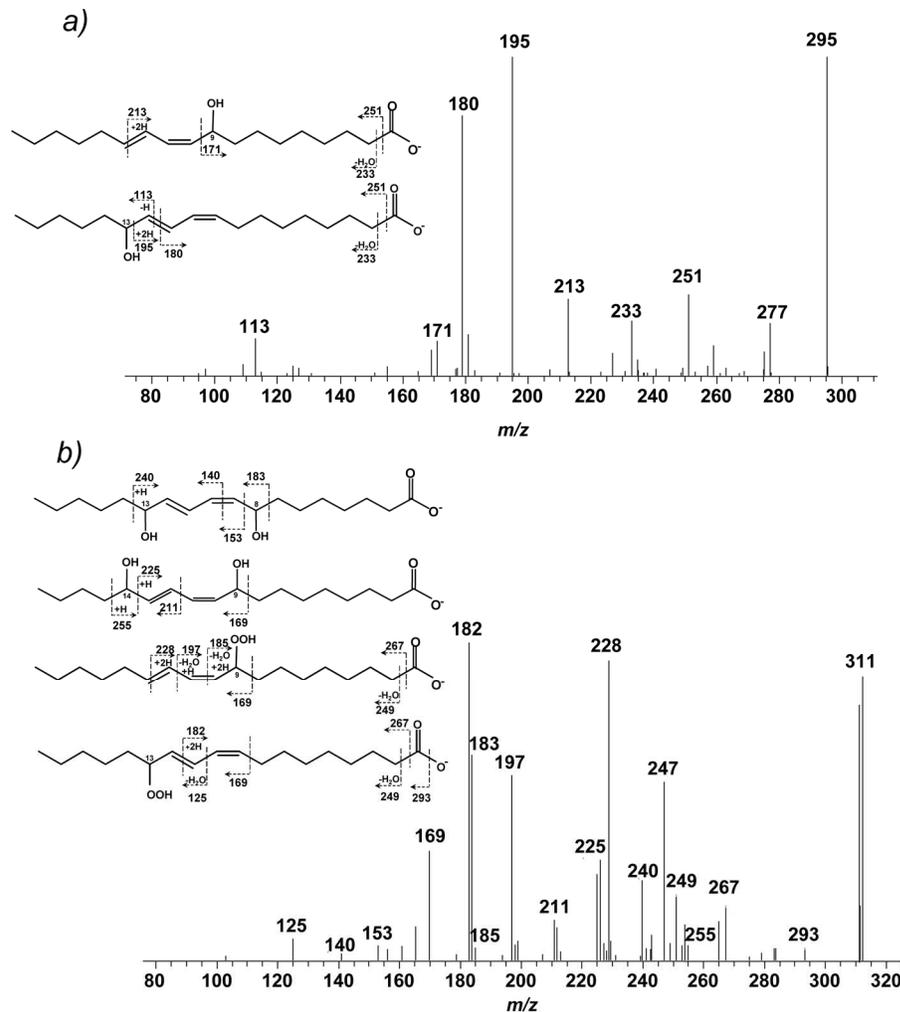


Figure S4. **Identification of oxygenated fatty acids in CL obtained from the lung of mice exposed to SWCNT.**

- a) Typical MS/MS spectrum of oxygenated $C_{18:2}$ with m/z 295 after hydrolysis of CL by PLA_2 . Fragmentation patterns of molecular ions with m/z 295 (insert). Molecular ion with m/z 295 was identified as two overlapping oxygenated molecular species of $C_{18:2}$: 13-OH- $C_{18:2}$ and 9-OH- $C_{18:2}$.
- b) Typical MS/MS spectrum of oxygenated $C_{18:2}$ with m/z 311 after hydrolysis of CL by PLA_2 . Fragmentation patterns of molecular ions with m/z 311 (insert). Molecular ions with m/z 311 was identified as four overlapping oxygenated molecular species of $C_{18:2}$: 13-OOH- $C_{18:2}$ and 9-OOH- $C_{18:2}$, 13,-8-di-OH- $C_{18:2}$ and 9,-14-di-OH- $C_{18:2}$.

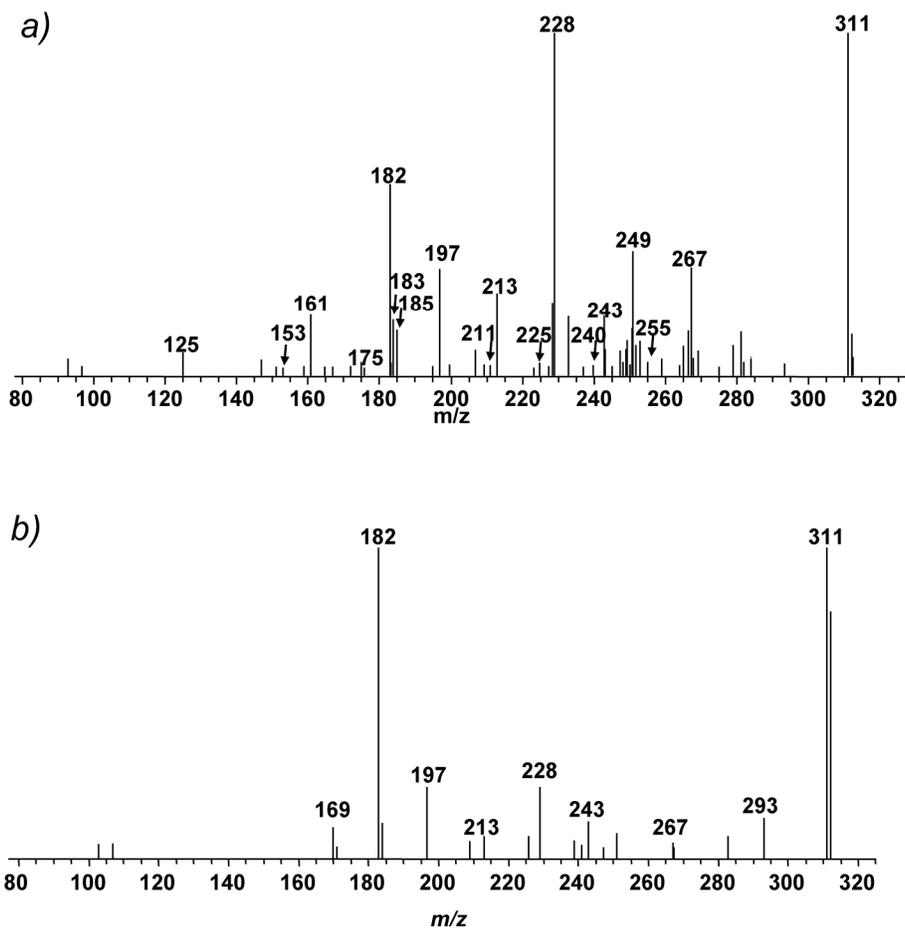


Figure S5. Typical MS/MS spectra of oxygenated $C_{18:2}$ with m/z 311 after hydrolysis of PS (a) and PI (b) by PLA_2 .

Oxygenated $C_{18:2}$ obtained after hydrolysis of PS was represented by four oxygenated molecular species and identified as 13-OOH- $C_{18:2}$ and 9-OOH- $C_{18:2}$, 13,-8-di-OH- $C_{18:2}$ and 9,-14-di-OH- $C_{18:2}$. Hydrolysis of PI revealed the presence of two mono-hydroperoxy-molecular species of $C_{18:2}$: 13-OOH- $C_{18:2}$ and 9-OOH- $C_{18:2}$.