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研究課題名（和文）精密な脂質分析を用いるヒト肺と皮膚細胞に対する環境濃度オゾンの影響に関する研究

研究課題名（英文）Study on the Influence of Environmental Concentration of Ozone on Lung and Skin Cells by Lipid Analysis

研究代表者
喬　琳（Qiao, Lin）

北海道大学・保健科学研究院・助教

研究者番号：３０８２１５２４

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研究成果の概要（和文）：極めて高い酸化反応性を持つオゾンは、人間にとって普遍的な環境毒性物質である。ヒト呼吸気道および皮膚組織は、この酸化性汚染物質に最も直接的に曝される二つの主要な器官である。オゾンは脂質過酸化の生成を介して肺に炎症を誘発すると仮定されている。本研究は、高分解能のオービトラップ型高速液体クロマトグラフィー質量分析装置を用いて、新規の精密な脂質解析方法の確立に成功した。これを利用して、日常大気中で観測された平均濃度と同様の低レベルのオゾンに曝露した脂質の軽微な酸化変性を検出できて、分子レベルでヒト肺サーファクタント脂質と肺細胞の酸化過程についてを検討した。

研究成果の学術的意義や社会的意義
本研究で確立した高分解能かつ高感度な酸化脂質の測定分析方法は、ヒトの肺細胞に対する環境濃度オゾンの影響を研究する有効な方法である。それを用いて、日常大気中で観測された平均濃度と同様の低レベルのオゾンによるモデル肺サーファクタントとヒト肺細胞の安定性と機能性への影響を検討できるようになった。これらの基礎知見を活かし、極低濃度のオゾンによる健康被害及びその防止策に重要な指針を提示できると考えている。

研究成果の概要（英文）：Ozone, with extremely high oxidative reactivity, is a common environmental toxicant to the individuals. The respiratory airways and skin tissues are two major organs most directly exposed to this oxidant pollutants. Ozone is hypothesized to initiate intracellular oxidative stress and induce the inflammation in the lung and skin through the formation of oxidized lipids. In the present study, in order to study the lipid oxidation during the exposure to the low-level ozone, which is similar to the average concentration observed in ambient air, we successfully establish a novel and sensitive lipid analysis method by high performance liquid chromatography combined with high-resolution orbitrap tandem mass spectrometry. The ozonized lipids of the lung surfactants and the lung cells were successfully identified and quantified. It helps us to further understand the reaction mechanism between the lipid in the lung and the low-level ozone in the air at a molecular level.

研究分野：化学

キーワード：low-level ozone　ozonation　lipid oxidation　A549 cell　lung surfactant　lung damage　orbitrap LC-MS/MS

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Ozone is famous for preventing harmful ultraviolet radiation to reach the earth at the high altitude, but in the ambient air it is a common environmental toxicant to human health, since it has extremely high oxidative reactivity. The effect of ambient ozone on the human health has always been ignored due to its low concentration in the air, ca. a few tens of ppb. Plenty of studies indicated ozone can initiate and aggravate the disease in the lung and the skin, especially in children and older adults.

Ozone is hypothesized to initiate intracellular oxidative stress and induce the inflammation in the lung through the formation of lipid peroxidation. Therefore, to analyze oxidized lipids is an effective way to study the influence of ambient-level ozone on the human lung. However, previous analysis on the oxidized lipids mainly focused on the detection of the major products by employing rather high concentrations of ozone (300–2000 ppb). The condition of the high concentration is easy to operate but has the possibility to miss the potential oxidation products which may be the important mediator in the bio-system. To profiling all the oxidation products under a low level of ozone is still an analytical challenge, which requires the development of a high-sensitive and high-resolution approach with the ability the detect the complex and precise changes of the lipids during the oxidation reaction.

２．研究の目的

In this study, we aim to establish a novel liquid chromatography–mass spectrometry (LC/MS)-based lipid analysis method to detect the oxidized lipid in the pulmonary surfactant and the human lung cells during the exposure to low level of ozone. We want to answer the questions: for the lipids present in pulmonary surfactant and lung cells, (1) what kind of oxidized lipid will be generated (2) how the low-level ozone influences the physical-chemical properties of the lung surfactant membrane and the biological functions of the lung cell.

３．研究の方法

(1) The novel LC/MS-based analysis method was established by using a high-performance liquid chromatography combined with high-resolution orbitrap tandem mass spectrometry (HPLC-Orbitrap MS).

(2) The lung surfactant membrane was made by spreading a lipid monolayer on the water surface. After exposing to the ozone (10-70 ppb), its oxidation products in the low-level ozone were systematically studied by the LC/MS.

(3) The adenocarcinomic human alveolar basal epithelial cell line (A549) was chosen as the model and cultured in a home-made ozone exposure system, which was setup by combining a self-made ozone pump system, a sensitive ozone sensor and an incubator together. After exposing to the ozone (10-100 ppb), all the lipids of the cell were extracted and further studied by the LC/MS.

４．研究成果

In the present study, a LC/MS-based analysis method was successfully established and applied to study the reaction of the model lung surfactant membrane and the lung cell with low-level of ozone (10-100 ppb).

(1) Study on the Oxidation of the Model Lung Surfactant Membrane

① The Identification of the Ozonized Products

The model membrane was composed by the major lipids of the lung surfactant including 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), and palmitoyl-2-(9'-oxo-nonanoyl)-*sn*-glycero-3-phosphoglycerol (POPG). After exposing the model membrane to the low-level ozone (10-70 ppb), we found the saturated lipids DPPC is very stable, but the unsaturated lipids POPC and POPG are unstable. An aldehyde product, 1-palmitoyl-2-(9'-oxo-nonanoyl)-*sn*-glycero-3-phosphocholine (POnPC), an acid product 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (PAzPC) and a secondary ozonide (SOZ) can be identified as the ozonized products of the POPC (Figure 1). Similarly, palmitoyl-2-(9'-oxo-nonanoyl)-*sn*-glycero-3-phosphoglycerol (POnPG), the 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphoglycerol (PAzPG) and corresponding SOZ can be identified as the ozonized products of POPG.

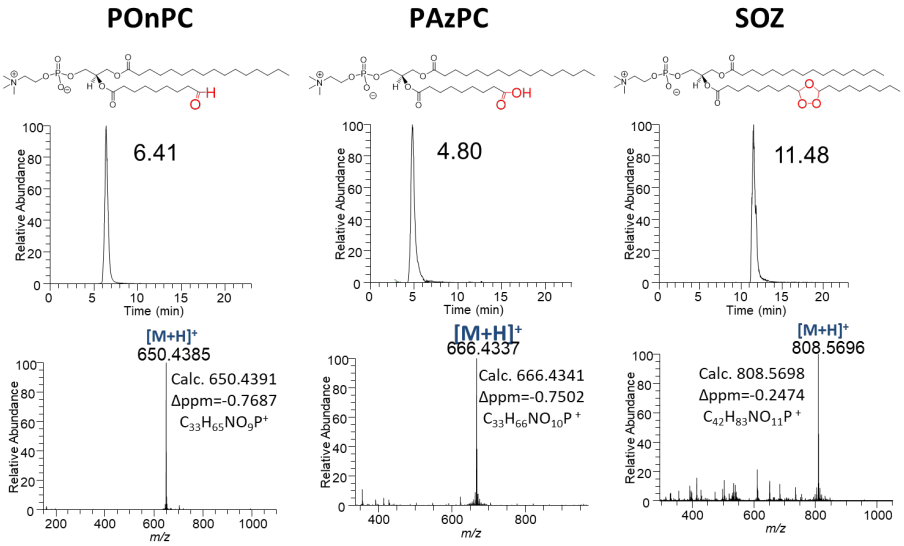
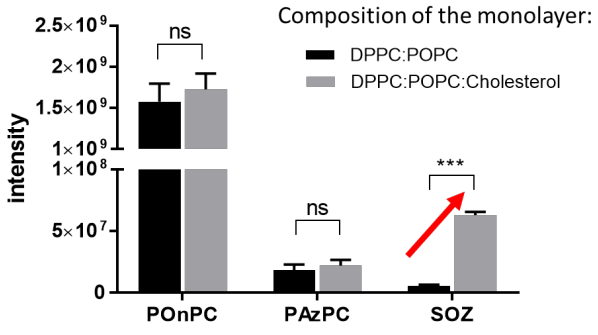


Figure 1. The identification of ozonized products of POPC in the model lung surfactant monolayer by LC/MS.

② The Influence of the Cholesterol

Cholesterol another important lipid in the lung system. By mixing the cholesterol with the



***p < 0.001, ns: not significant (n = 4)

Figure 2. Comparison of the amount of the ozonized lipid between the mixed monolayer of DPPC:POPC and DPPC:POPC:Cholesterol.

phospholipid, we found the existence of the cholesterol in the model lipid monolayer can increase the amount of the secondary ozonide (Figure 2). Because the cholesterol can affect the packing density of the lipids in the monolayer, it indicates the physical-chemical properties of the lipid monolayer can strongly affect the reaction mechanism and thus change the amount of the products.

(2) Study on the Lung Cell

An adenocarcinomic human alveolar basal epithelial cell line (A549) was cultured in a home-made ozone exposure system and exposed to low-level ozone with a concentration of 0-100 ppb. By comparing with the cell in the normal culture condition, we found even in such a low concentration of ozone, the viability of cells shows only gradually decrease, especially after the long-time exposure of 12-72 hours. Also, for the first time, three kinds of secondary ozonide of the lipids with the phosphatidylcholine (PC) head group, 32:1 PC, 34:1 PC (POPC), 36:1 PC, were identified as the products of the lung cell in the low concentration of ozone (Figure 3).

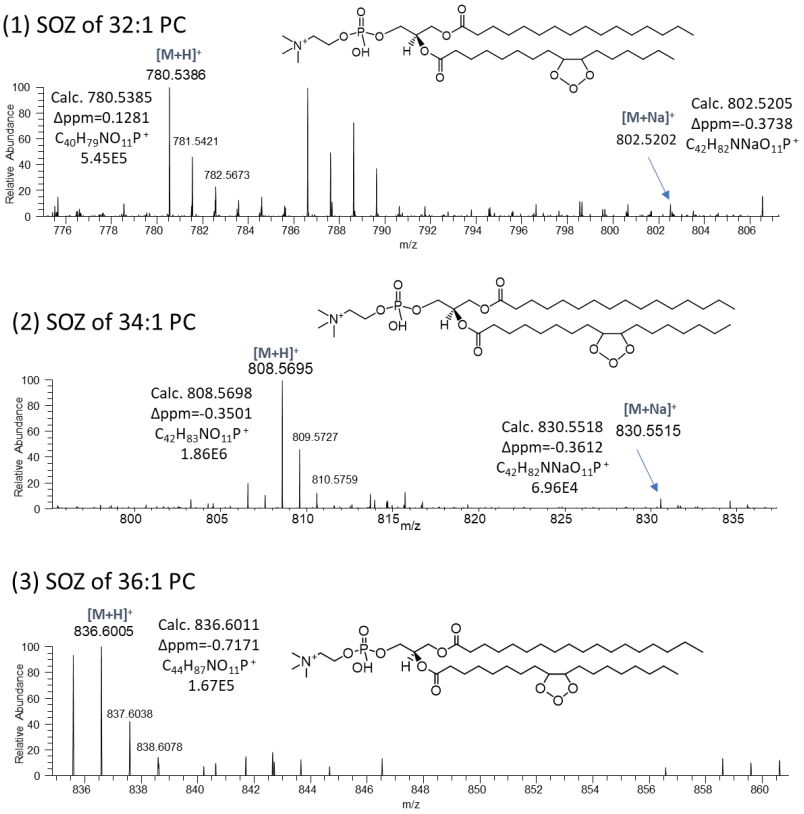


Figure 3. The identification of the SOZ of PC species in the lung cell after exposing to the low-level ozone by LC/MS.

Moreover, we found the amount of ozonized products their amount has a strong relationship with the relative humility of the air. The higher of the humility, the faster yield of the products. It indicates the humidity increased the reaction ability of the ozone with the lipids.

In summary, by using our self-established LC/MS analysis methods, we confirmed the low-level ozone can react with lipids of the human lung surfactant membrane and the lung cells. In the future, the oxidized products found in this research will be further studied to figure out their biological effect on the cell and human bodies.

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掲載論文のDOI（デジタルオブジェクト識別子） 10.1021/acs.langmuir.8b02676	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考