EP-283

Phosphatidylinositol-rich liposomes prevent oxidative stress induced by diesel particulate matter through suppression of activation of aryl hydrocarbon receptor (AhR)

Yoko Niki[1], Hiromi Onouchi[2]; Hiroki Kotera[2]; Yukihiro Ohashi[2*]

[1] Department of Health and Bio-Pharmaceutical Sciences, Mukogawa Women's University, Hyogo, Japan

[2] Cosmetic ingredients division, Nippon Fine Chemical Co., Ltd., Hyogo, Japan

Mukogawa Women's University

Your Beauty Partner
Nippon Fine Chemical

Introduction

Phospholipids are widely used as raw materials in emulsifiers and liposomes. However, few developments have been made for materials focused on the physiological activities of phospholipids. In the present study, we focused on **phosphatidylinositol** (**PI**; Fig. 1), a biological phospholipid.

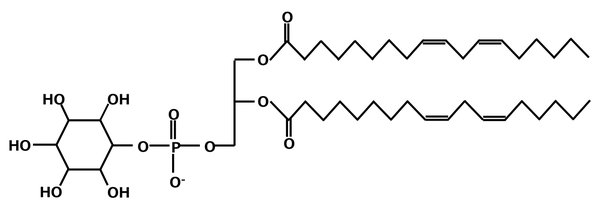


Fig. 1 Representative structure of PI

Many studies have indicated that **air pollutants accelerate the sings of skin aging,** such as the formation of pigment spots and wrinkles [1, 2]. Since skin is the outermost part of the body, it is susceptible to the effect of reactive oxygen species (ROS) generated by air pollutants, such as benzopyrene, a potent aryl hydrocarbon receptor (AhR) activator and a component of diesel particulate matter (DPM). AhR activation increases the expression of the detoxifying enzyme cytochrome P450 family 1 subfamily A polypeptide 1 (CYP1A1), which produces ROS [3]. Therefore, oxidative stress in cells is involved in skin aging caused by air pollutants. We previously reported that PI liposomes reduce oxidative stress in keratinocytes by activating nuclear factor erythroid 2-related factor 2 (Nrf2) signaling [4, 5]. In the present study, we examined the preventive effects of PI liposomes on DPM-induced skin aging.

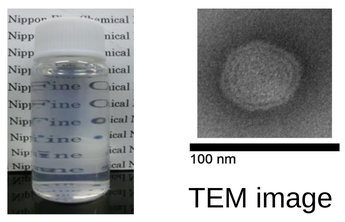
Materials and Methods

Preparation of PI-rich liposomes (PI liposomes)

Lecithin, sterol, and phospholipid containing PI were dissolved in an organic solvent. Then, the solvent was removed to obtain a PI complex. PI liposomes were prepared by dispersing 0.2% of the PI complex in water containing glycerin and pentylene glycol at 70-75 °C using a homomixer at 5000 rpm.

Table 1 Formulation of PI liposomes

	Formulation (%)
PI complex	0.2
Glycerin	3.0
Pentylene glycol	2.0
Phenoxyethanol	0.2
Water	Up to 100



TEM image

Fig. 2 PI liposomes

Evaluation using a cell co-culture system

Fig. 3 shows design of the cell co-culture system. The human epidermal keratinocyte cell line (HaCaT) seeded in the cell inserts (MCHT24H48; Millipore) were cultured in PI liposome-containing medium. After 24 h, HaCaT keratinocytes were co-incubated with freshly cultured HaCaT keratinocytes or human dermal fibroblasts (NHDFs) in a 24-well cell culture plate and exposed to 200 μg/mL DPM (NIST 1650b, NIST).

Intracellular ROS Intracellular ROS levels in cultured HaCaT keratinocytes and NHDFs cultured in the cell culture plate were evaluated using 2',7'- dichlorofluorescein diacetate (H_2DCFDA, Merck).

mRNA expression The gene expressions of *CYP1A1* in HaCaT keratinocytes and *MMP-1* and *COL1A1* in NHDFs in the cell culture plate were evaluated by quantitative reverse transcription polymerase chain reaction (RT-qPCR) method.

Immunofluorescence staining of AhR and type I collagen Intercellular distribution of AhR in HaCaT keratinocytes and type I collagen fiber in NHDFs co-cultured with DPM-exposed HaCaT keratinocytes was evaluated via immunofluorescence staining. Fluorescence images were obtained using a fluorescence microscope (Keyence, Osaka, Japan).

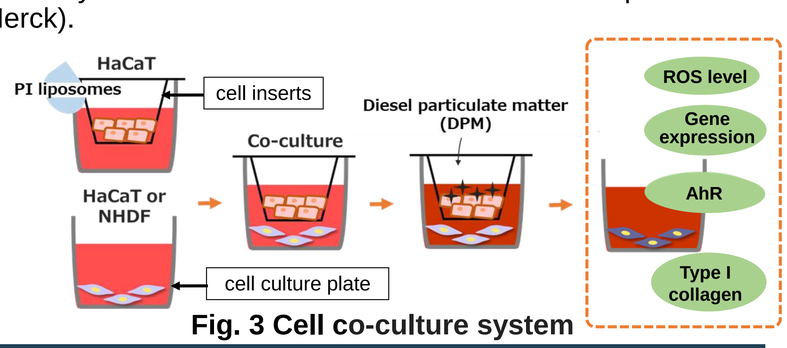
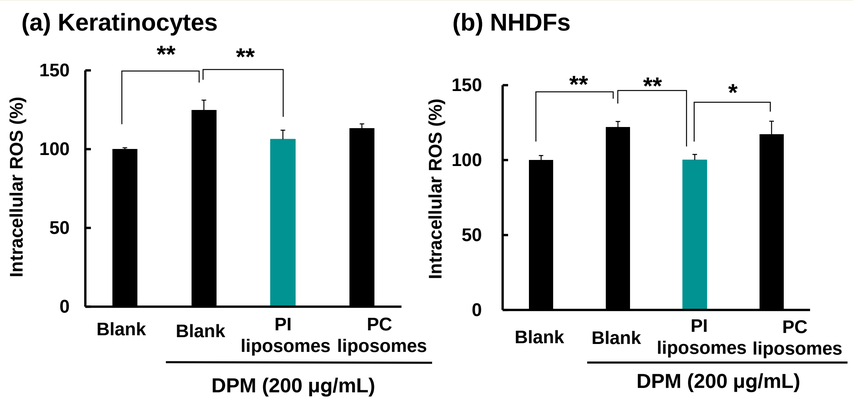


Fig. 3 Cell co-culture system

Results and Discussion

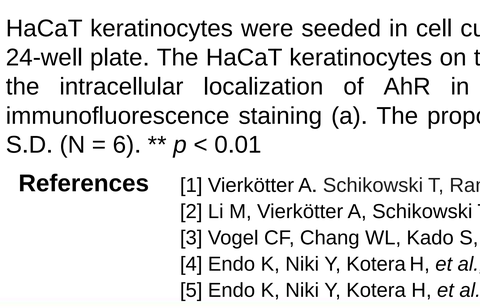
Fig. 4 PI liposomes suppressed DPM-induced increase in ROS production.



HaCaT keratinocytes were seeded in cell culture inserts and co-cultured with HaCaT keratinocytes (a) or NHDFs (b) seeded on a 24-well plate. HaCaT keratinocytes on the cell culture insert were treated with 200 μg/mL DPM for 24 h, and intracellular ROS production on the 24-well plate was determined. Mean ± S.D. (N = 4) ** $p < 0.01$, * $p < 0.05$

PC liposome: contains almost no PI; used as control liposome. Main component is phosphatidylcholine (PC).

Fig. 5 PI liposomes suppressed DPM-induced increase in *CYP1A1* expression.

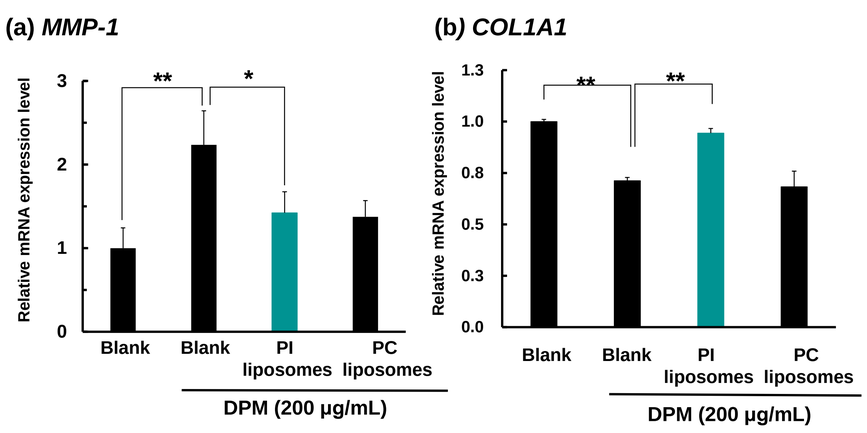


HaCaT keratinocytes were seeded in cell culture inserts and co-cultured with HaCaT keratinocytes seeded on a 24-well plate. The HaCaT cells on the cell culture insert were treated with 200 μg/mL DPM for 24 h, and the expression level of the *CYP1A1* gene in HaCaT keratinocytes on the 24-well plate was measured by RT-qPCR. Mean ± S.D. (N = 3) *** $p < 0.001$, * $p < 0.05$

Fig. 6 PI liposomes suppressed DPM-induced nuclear translocation of AhR.

HaCaT keratinocytes were seeded in cell culture inserts and co-cultured with HaCaT keratinocytes seeded on a 24-well plate. The HaCaT keratinocytes on the cell culture insert were treated with 200 μg/mL DPM for 24 h, and the intracellular localization of AhR in HaCaT keratinocytes on the 24-well plate was detected by immunofluorescence staining (a). The proportion of AhR localized to the nucleus was determined (b). Mean ± S.D. (N = 6). ** $p < 0.01$

Fig. 7 Effect of PI liposomes on gene expression of *MMP-1* and *COL1A1*.



HaCaT keratinocytes were seeded in cell culture inserts and co-cultured with NHDFs seeded on a 24-well plate. HaCaT keratinocytes on the cell culture insert were treated with 200 μg/mL DPM for 24 h. The gene expression levels of *MMP-1* (a) and *COL1A1* (b) in NHDFs were detected by RT-qPCR. Mean ± S.D. (N = 3) ** $p < 0.01$, * $p < 0.05$

Fig. 8 Effect on type I collagen production in NHDFs.

Immunostaining of type I collagen fibers of fibroblasts co-cultured with HaCaT keratinocytes exposed to DPM for 7 d. Representative images of type I collagen fiber formation are shown.

The production of type I collagen was reduced in fibroblasts co-cultured with DPM-exposed HaCaT keratinocytes than in untreated cells. However, this reduction was suppressed in cells cultured with PI liposomes.

Conclusions

- **PI liposomes suppressed AhR signaling activated by DPM and attenuated *CYP1A1* expression and ROS production (Fig. 4, 5, and 6).**
- **The decrease in type I collagen fiber formation caused by DPM was restored by PI liposomes (Fig. 7 & 8).**

Our results suggest that PI liposomes are useful as an antipollution ingredient that can alleviate oxidative stress caused by air pollutants and prevent skin aging.

References

[1] Vierkötter A. Schikowski T, Ranft U, *et al.,* **(2010)** *J Invest Dermatol.* 130:2719-26.
[2] Li M, Vierkötter A, Schikowski T, *et al.,* **(2015)** *J Dermatol Sci.* 79:148-54.
[3] Vogel CF, Chang WL, Kado S, *et al.,* **(2016)** *Environ Health Perspect.* 124:1071-83.
[4] Endo K, Niki Y, Kotera H, *et al.,* **(2019)** *In Proceeding of the 25th IFSCC conference*
[5] Endo K, Niki Y, Kotera H, *et al.,* **(2020)** *K-beauty Science,* 13:109-123.