J. Soc. Cosmet. Scientists Korea
Vol. 30, No. 2, September 2004, 247-251

인체각질형성세포에서 Fructose 1,6-diphosphate의 자외선에 의해 유도되는 Cyclooxygenase-2 and Matrix Metalloproteinases의 발현억제기전

안 수 미† · 김 지 현 · 이 병 곤 · 이 수 환* · 장 이 섭

태평양기술연구원 피부과학연구소, *아주대학교 의과대학 생리학교실

Fructose 1,6-diphosphate Prevents Cyclooxygenase-2 and Matrix Metalloproteinases Expression by Inhibition of UVB-induced Signaling Cascades in HaCaT Keratinocytes

Soo Mi Ahn†, Ji Hyun Kim, Byeong Gon Lee, Soo Hwan Lee*, and Ih Seoup Chang

Skin Research Institute, Amore Pacific R&D Center, 314-1, Bora-ri, Giheung-eup, Yongin-si 449-729, Korea
*Department of Physiology, School of Medicine, Ajou University

요 약: 자외선은 피부에 염증반응이나 광노화와 같은 다양한 반응을 야기시킨다고 알려져 있다. 특히 자외선에 의해 손상을 받은 피부는 콜라겐의 양이 감소되어 있는데, 이는 자외선에 의해 피부 내에서 콜라겐을 분해하는 효소(MMP, matrix metalloproteinases)의 양이 증가하기 때문이라고 알려져 왔다. 또한 자외선에 의해 피부에서 염증반응이 유발되는데, 이러한 반응은 프로스타글란딘이라는 물질에 의해 매개되며, 이 프로스타글란딘에 의해서도 MMP가 증가한다고 알려져 왔다. 본 연구에서는 6개월간의 임상실험을 통해 광노화된 피부에서 주름형성억제효능이 뛰어난 FDP (fructose 1,6-diphosphate)의 작용기전을 인체각질형성세포를 이용하여 연구하였다. 인체각질형성세포에 자외선을 조사할 경우 프로스타글란딘, COX-2 (cyclooxygenase-2), MMPs의 활성이 증가하는 것을 확인하였으며, 이는 FDP의 처리에 의해 감소되었다. 이러한 효과는 자외선에 의해 인체각질형성세포에서 발생하는 신호전달과정을 억제함으로써 일어나는 효과임이 증명되었다. 따라서, FDP는 자외선에 의해 일어나는 세포 내 신호전달과정을 억제하며, 이로 인해 야기되는 프로스타글란딘, COX-2, MMPs의 증가를 억제함으로써 피부의 광노화를 억제할 수 있는 원료로 여겨진다.

Abstract: UV radiation exerts various influences in the skin, including photoaging and inflammation (1). The MMPs (Matrix metalloproteinases), which are induced by UV irradiation, can degrade matrix proteins, and these results in a collagen deficiency in photodamaged skin that leads to skin wrinkling. It has been known that the production of PGE_2 stimulates MMPs expression, and inhibits procollagen (2). Thus, it is possible that the induction of MMPs and the inhibition of matrix protein synthesis by UV-induced PGE_2 may play some role in UV-induced collagen deficiency in photoaged skin. Fructose-1,6-diphosphate (FDP), a glycolytic metabolite, is reported to have cytoprotective effects against ischemia and postischemic reperfusion injury of brain and heart, presumably by augmenting anaerobic carbohydrate metabolism (3). And also, FDP significantly prevent skin aging by decreasing facial winkle compared with vehicle alone after 6 months of use. We studied the mechanism of anti-aging effect of FDP on UVB-irradiated HaCaT keratinocyte model. FDP has protective role in UVB injured keratinocyte by attenuating prostaglandin E_2 (PGE_2) production and COX-2 expression. And FDP also suppressed UVB-induced MMP-2 expression. Further, to delineate the inhibition of UVB-induced COX-2 and MMPs expression with cell signaling pathways, treatment of FDP to HaCaT keratinocytes resulted in marked inhibition of UVB-induced phosphorylation of ERK1/2, JNK. It also prevents UV induced NFB translocation, which are activated by cellular inflammatory signal. Our results indicate that FDP has protecting effects in UV-injured skin aging by decreasing UVB-induced COX-2 and MMPs expression, which are possibly through blocking UVB-induced signal cascades.

Keywords: *FDP, UV irradiation, aging, COX-2*

† 주 저자 (e-mail: smahn@amorepacific.com)

1. Introduction

Aging of skin is a complex biological phenomenon consisting of two components; intrinsic aging and photoaging caused by environmental exposure, primarily UV light. However, decreased metabolic function and accumulated oxidative damage induced by ROS (reactive oxygen species) are responsible for cutaneous inflammatory disorder and skin aging. It is well known that UV irradiation induces the formation of ROS in cutaneous tissue.

FDP completely inhibits generation of oxygen free radicals by stimulated neutrophils. It is well documented that UV irradiation increases oxidative stress in irradiated tissue and oxidant components play an important role in the signaling events leading to gene activation after UV irradiation. Recently, we showed that FDP reduced UVB-induced increase in cellular ROS level although it did not show direct radical scavenging effect in the experiment using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and significantly prevent skin aging by decreasing facial winkle compared with vehicle alone after 6 months of use.

In this study, we examined the mechanism of the anti-aging effect of FDP in UVB-induced HaCaT keratinocyte model.

2. Materials and Methods

2.1. Cell Cultures and UVB Irradiation

HaCaT keratinocytes were grown in DMEM medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum and 1% antibiotic and antimycotic solution. For experiments, cells were maintained in DMEM supplemented with 1% fetal bovine serum (FBS) for indicating time.

UVB radiation was provided by a bank of Sankyo Denki G15T8E, a fluorescent bulb emitting 280~320 nm wave with a peak at 313 nm. UVB was delivered with the dose of 30 mJ $cm^{2\ -1}$ and the irradiance was monitored with an IL1700 radiometer (International Light Inc., Newburyport, MA, USA). Before UV exposure the cells were washed twice in phosphate-buffered saline (PBS), and the cells were covered with PBS during UV irradiation. After the UV exposure, fresh culture medium was added and the cells and media were harvested at the indicated time points for further experiments.

2.2. PGE_2 Mesurement

Levels of PGE_2 were determined using enzyme immunoassay kit from Cayman chemical (Ann Arbor, MI, USA).

2.3. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The total RNA was isolated from the cell samples using a Trizol reagent (Gibco BRL, Gaithersburg, MD) following the manufacturer's instruction. RT-PCR was performed using One Step RNA PCR kit (Takara, Shiga, Japan). PCR primers were produced by custom oligonucleotide synthesis service (Bioneer, Korea). Target gene mRNA levels were quantified based on standard and normalized to GAPDH (control) mRNA level.

2.4. Determination of MMP-2 and 9 Activities

The activities of MMP-2 and -9 were measured by zymogram protease assays. Eight percent SDS polyacrylamide gels (0.75 mm thick, acrylamide/bis-acrylamide = 30/1.2) containing 0.1% gelatin was used for electrophoresis. Samples (containing 10 mg protein) were prepared with standard SDS-gel-loading buffer containing 0.01% SDS. Reducing agents were not included, and samples were not boiled prior to loading. After electrophoresis, the gel was washed twice with 100 mL distilled water containing 2% Triton X-100 for 30 min at room temperature to remove SDS. The gel was then incubated in 100 mL reaction buffer (40 mmol/L Tris-HCl, pH 8.0, 10 mmol/L $CaCl_2$, 0.02% NaN_3) for 16 h at 37℃, stained with Coomassie brilliant blue R-250 and destained with methanol:acetic acid:water (50 : 75 : 875, v/v/v).

2.5. Immunoblotting

Immunoblot analysis for detection of phosphorylated protein for ERKs, JNKs and p38 kinase were carried out using the phosphospecific antibodies (Cell signaling, Beverly, MA). And human COX-2 antibody was purchased from Calbiochem.

2.6. Electrophoretic Mobility Shift Assay (EMSA)

NFkB double standard oligonucleotides (Promega, Madison, WI) were end-labeled with [-^{32}P]-ATP (Amersham, Arlington Heights, IL). One microliter (30,000 cpm) of labeled probe was incubated with 3 $\mu\ell$ of nuclear extract concentration, 5 μg) and binding buffer (15 mM Tris-HCl, pH 7.5, 7.5% glycerol, 75 mM sodium chloride, 1.5 mM EDTA, 1.5 mM dithiothreotol, 0.5 mM phenylmethylsulfonyl fluoride, 0.3% NP-40, and 20 μg bovine serum albumin), final volume 25 $\mu\ell$ for 30 min at 25℃. The mixtures were resolved in 4 or 6% nondenaturing polyacrylamide gel containing 2.5% glycerol in 0.5× TBE buffer at 4℃. The gels were dried and visualized by autoradiography.

3. Results

3.1. FDP Attenuates UVB-induced PGE_2 Production and COX Expression

FDP reduced the accumulated levels of PGE_2 in the media in a dose dependent manner when added to the culture media immediately after irradiation (Figure 1). UVB-induced PGE_2 production was also significantly inhibited by NS-398 (1 μM), a COX-2 selective inhibitor, and NAC (20 mM), an antioxidant. A single UVB exposure (30 mJ $cm^{2\ -1}$) induced COX-2 expression in HaCaT cells. The increase in COX-2 expression stimulated by UVB was markedly reduced by FDP (Figure 2).

3.2. FDP Attenuates UVB-induced MMP-2 Expression

A single UVB exposure (30 mJ $cm^{2\ -1}$) induced MMP-2 gene expression in HaCaT cells. The maximal induction of MMP-2 mRNA was observed in 2 h post irradiation. The increase in MMP-2 expression stimulated by UVB was markedly reduced by FDP (Figure 3). We then examined MMP-2 and MMP-9 secretion with or without UVB irradiation. As shown in Figure 4, the intensity of the MMP-2 band increased 48 to 72 h after irradiation and FDP reduced MMP-2 secretion in dose dependent manner.

3.3. FDP Prevents UVB-induced Phosphorylation of MAPK in HaCaT Cell

The MAPK proteins are important upstream regulators

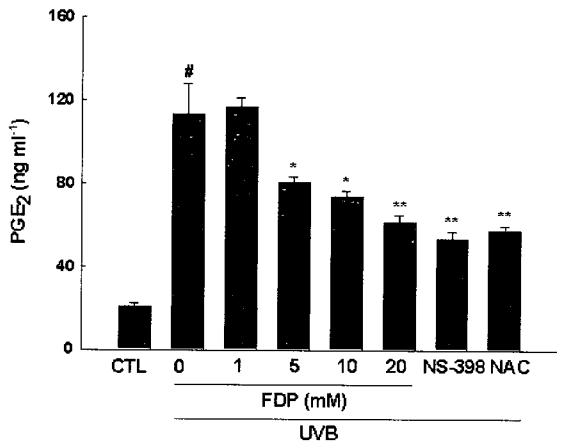

Figure 1. Effect of FDP on UVB-induced PGE_2 production.

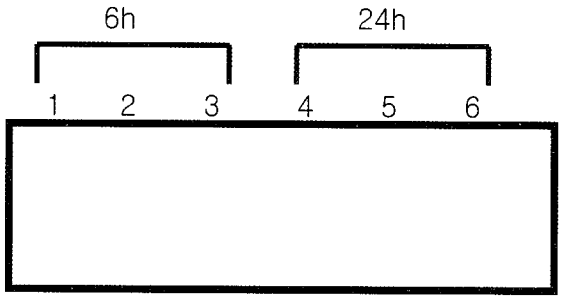

Figure 2. Effect of FDP on UVB-induced COX-2 expression. Lane 1,4; control, lane 2,5; UVB, lane 3,6; UVB + FDP (10 mM).

of transcription factor activities and their signaling is critical to the transduction of a wide variety of extracellular stimuli into intracellular events (4). Therefore, we were interested in examining the effects of FDP on UVB-induced phosphorylation of MAPK proteins in HaCaT cells. UVB-induced phosphorylation of ERK1/2 was observed 10 min after irradiation and FDP treatment markedly inhibited UVB-induced phosphorylation of ERK1/2 (Figure 5). UVB-induced phosphorylation of JNK was also observed and FDP inhibited phosphorylation of JNK (Figure 5), but FDP did not prevent UVB- induced p38 phosphorylation (data not shown).

3.4. FDP Prevents UVB-induced Translocation of NFκB in HaCaT Cells

UV irradiation to cell induced compensatory activations of multiple intracellular signaling pathway (5). This includes UV-induced signaling via the EGFR and IGFI-R to the PI3K, MAPK pathways as well as FAS-R

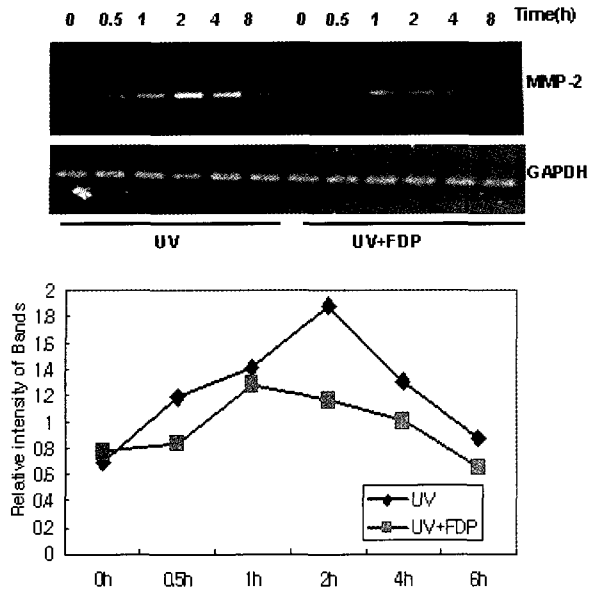

Figure 3. Effect of FDP on UVB-induced MMP-2 expression.

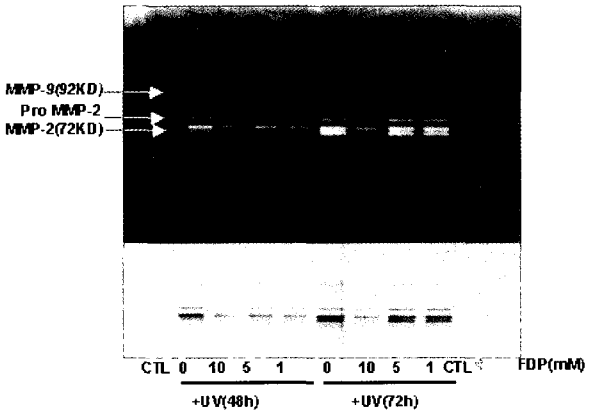

Figure 4. Effect of FDP on UVB-induced MMP-2 and 9 activities.

and TNF-R signaling to pro-caspases and NFκB. FDP prevented UVB-induced phosphorylation of MAPKs, we were interested in the effect of FDP on UVB-induced translocation of NFκB. UVB-induced translocation of NFκB was observed 30 min after irradiation and FDP treatment markedly inhibited UVB-induced nuclear translocation of NFκB (Figure 6).

4. Conclusion

Fructose-1,6-diphosphate (FDP), a glycolytic metabolite,

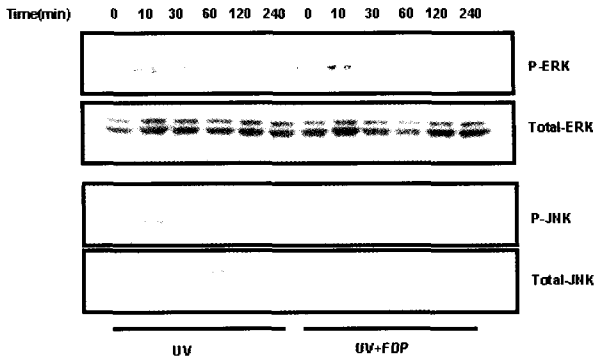

Figure 5. Effect of FDP on UVB-induced phosphorylation of MAPK.

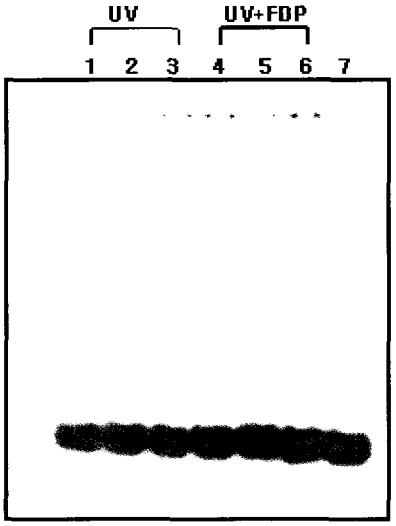

Figure 6. Effect of FDP on UVB-induced NFκB translocation. Lane1,4; Control, lane 2,5; 30 min incubation after UVB irradiation, lane 3,6; 1 h incubation after UVB irradiation, lane 7; cold.

is reported to have cytoprotective effects against UV irradiated skin. In this study, we examined the mechanism of this protective role of FDP in UVB irradiated HaCaT keratinocytes.

In this research, FDP attenuated UVB-induced PGE_2 production. And FDP also suppressed UVB-induced COX-2 and MMP-2 expression. Further, to delineate the inhibition of UVB-induced COX-2 and MMPs expression with cell signaling pathways, treatment of FDP to HaCaT keratinocytes resulted in marked inhibition of UVB-induced phosphorylation of ERK1/2, JNK. It also prevents UV induced NFκB translocation, which are activated by cellular inflammatory signal.

COX-2 and MMPs are reported as an effective target for the regulation of UV-induced skin disorders, FDP could be use for the skin protective materials against UV-induced skin damage.

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