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Fructose-1,6-diphosphate attenuates prostaglandin E_2 production and cyclo-oxygenase-2 expression in UVB-irradiated HaCaT keratinocytes

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1 Fructose-1,6-diphosphate (FDP), a glycolytic metabolite, is reported to ameliorate inflammation and inhibit the nitric oxide production in murine macrophages stimulated with endotoxin. It is also reported that FDP has cytoprotective effects against hypoxia or ischaemia/reperfusion injury in brain and heart. However, underlying mechanisms of its various biological activities are not completely understood.

2 In this study, we examined the effects of FDP on UVB-induced prostaglandin production in HaCaT keratinocytes.

3 Ultraviolet B (UVB, 280–320 nm) irradiation (30 mJ cm^{-2}) increased prostaglandin E_2(PGE_2) production, which was significantly decreased by FDP in a concentration dependent manner. NS-398, a cyclo-oxygenase-2 (COX-2) selective inhibitor completely inhibited UVB-induced PGE_2 production showing that COX-2 activity is responsible for the increase in PGE_2 production under our experimental conditions.

4 UVB irradiation increased total COX activity and COX-2 mRNA in HaCaT keratinocytes, which were significantly blocked by FDP in a concentration dependent manner.

5 N-acetylcysteine (NAC) significantly attenuated UVB-induced PGE_2 production, COX activity and COX-2 mRNA expression indicating oxidative components might contribute to these events.

6 FDP reduced UVB-induced increase in cellular reactive oxygen species (ROS) level although it did not show direct radical scavenging effect in the experiment using 1,1-diphenyl-2picrylhydrazil (DPPH). FDP preserved the cellular antioxidant capacity including catalase activity and GSH content after irradiation.

7 Our data obtained hitherto suggest that FDP may have a protective role in UVB-injured keratinocyte by attenuating PGE_2 production and COX-2 expression, which are possibly through blocking intracellular ROS accumulation.

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Keywords: FDP; HaCaT keratinocytes; UVB irradiation; cyclo-oxygenase-2; prostaglandin E_2; reactive oxygen intermediates

Abbreviations: COX, cyclo-oxygenase; DCFH-DA, 2,7′-dichlorofluorescein diacetate; DMEM, Dulbecco's minimum essential medium; DPPH, 1,1-diphenyl-2-picrylhydrazil; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); EDTA, ethylene-diamine tetraacetate; FCS, fetal calf serum; FDP, fructose-1,6-diphosphate; GSH, glutathione; NAC, N-acetylcysteine; NADP, nicotineamide disphosphate; PBS, phosphate-buffered saline; PGE_2, prostaglandin E_2; PPP, pentose phosphate pathway; ROS, reactive oxygen species; SOD, superoxide dismutase; UVB, ultraviolet B

Introduction

Ultraviolet (UV) irradiation induces cutaneous inflammation, characterized by erythema and oedema. UVB (280–320 nm) exposure increases the production of a variety of inflammatory mediators such as prostaglandins and leukotrienes (Greaves & Sondergaard, 1970; Hruza & Pentland, 1993). UVB-induced formation of proinflammatory eicosanoids has been suggested to be primarily due to enhanced activity and synthesis of phospholipase A_2 (Chen *et al*., 1996; Gresham *et al*., 1996; Kang-Rotondo *et al*., 1993). However, a recent study showed that enhanced cyclo-oxygenase-2 (COX-2) expression may also have an important role(s) in the increased prostaglandin synthesis and inflammatory reaction after UV irradiation (Isoherranen *et al*., 1999; Soriani *et al*., 1999). These reports parallel previous studies in which cyclo-oxygenase inhibitors suppressed UV-induced erythema (Farr & Diffey, 1986; Ibbotson *et al*., 1996; Wilgus *et al*., 2000). It has been suggested that the upregulation of COX-2 by UV radiation may contribute to photocarcinogenesis in the same way that COX-2 has been shown to contribute to colon cancer (Athar *et al*., 2001; Pentland *et al*., 1999). These

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reports suggest that COX-2 could be an effective target for the regulation of UV-induced skin disorders.

Fructose-1,6-diphosphate (FDP), a glycolytic metabolite, is reported to have cytoprotective effects against ischaemia and postischaemic reperfusion injury of brain and heart, presumably by augmenting anaerobic carbohydrate metabolism (Farias *et al*., 1990; Sola *et al*., 1996; Takeuchi *et al*., 1998). It has been also shown to mitigate the adverse effect of endotoxin by regulating the generation of nitric oxide (Edde *et al*., 1998). In addition, it has been demonstrated that FDP completely inhibits generation of oxygen free radicals by stimulated neutrophils (Sun *et al*., 1990). It is well documented that UVB increases oxidative stress in irradiated tissue and oxidant components play an important role in the signalling events leading to gene activation after UV irradiation (Tyrrell, 1996). Although mechanisms by which UVB upregulates COX-2 are not clearly defined, UVB-induced formation of reactive oxygen intermediates has been suggested to be involved (Isoherranen *et al*., 1999; Soriani *et al*., 1999). Based on these premises, we examined whether FDP could attenuate UVB-induced generation of reactive oxygen species and COX-2 expression in HaCaT keratinocytes.

Methods

Cell cultures

The experiments were performed with human skin keratinocyte cell line HaCaT which was kindly gifted from Dr Fusenig of the German Cancer Research Center (DKFZ). These keratinocytes were derived from normal skin of a male patient and spontaneously immortalized (Boukamp *et al*., 1988). They are aneuploid and form normal epidermis when transplanted to nude mice. Cells were grown in DMEM medium (Gibco, Grand Island, NY, USA) containing 10% fetal calf serum, antibiotics (penicillin, 100 U ml^{-1}; streptomycin, 100 $\mu g\ ml^{-1}$) and antimycotic (amphotericin B 0.25 $\mu g\ ml^{-1}$). For experiments, cells were maintained in DMEM supplemented with 1% fetal calf serum (FCS) for 18 h. FDP (1–20 mM), NS-398 (1 μM) and N-acetylcysteine (NAC, 20 mM) were added immediately after UV-irradiation.

In vitro *ultraviolet B irradiation*

UVB radiation was provided by a bank of Sankyo Denki G15T8E, a fluorescent bulb emitting 270 ~ 320 nm wave with a peak at 313 nm. Minimal erythema dose varies between 30–80 mJ, depending on the skin type and light source. Thus, UVB was delivered with a dose of 30 mJ cm^{-2} and the irradiance was monitored with an IL1700 radiometer (International Light Inc., Newburyport, MA, U.S.A.). Before UVB exposure the cells were washed twice in phosphate-buffered saline (PBS), and the cells were covered with PBS during UV irradiation. After the UBV exposure, fresh culture medium was added and the cells and media were harvested at the indicated time points for further experiments.

Determination of PGE_2 and COX activity

Spent media was removed at the indicated time and the accumulated levels of PGE_2 in the media were determined using enzyme immunoassay kit from Cayman chemical (Ann Arbor, MI, U.S.A.). To determine the COX activity, cells were harvested at the indicated time after irradiation, and rinsed with phosphate saline (PBS, 0.01 M, pH 7.4) prior to addition of fresh media containing arachidonic acid (10 μM). After incubation for 10 min at 37°C, media was removed and subjected to enzyme immunoassay for the measurement of PGE_2. Release of PGE_2 from exogenous arachidonic acid was taken as an index of COX activity (Fu *et al*., 1990).

Northern blot analysis

Total cellular RNA was isolated from cell cultures using the easy-BLUE™ RNA isolation kit (iNtRON, Seoul, Korea) and subjected to Northern analysis. Twenty micrograms of total RNA were fractionated on 1.2% agarose/formaldehyde gels in 1 × MOPS and transferred to nylon membranes (Hybond™, Amersham, Arlington Heights, IL, U.S.A.) and hybridized to a ^{32}P-labelled cDNA probe. The membranes were exposed to phosphor imaging plates and the amounts of COX-2 mRNAs were determined by a phosphor imaging plate scanner (Fujifilm, Tokyo, Japan, BS-5000). Human cDNA probe for COX-2 was a kind gift from Dr D.H. Hwang of Pennington Biomedical Research Center, Louisiana State University, U.S.A. ^{32}P-labelled cDNA probe for human COX-2 was prepared by random primed synthesis.

ROS determination

Cells grown on a glass-bottom dish were loaded with 5 μM 2,7′-dichlorofluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, OR, U.S.A.) in PBS. Cultures were incubated for 30 min at 37°C, washed three times with PBS, and the fluorescence signal of DCFH (Ex = 490 nm; Em = 510 nm), the oxidation product of DCFH-DA by free radicals, was analysed on the stage of a Nikon Diaphot inverted microscope equipped with a 100 W Xenon lamp. To minimize background signal caused by direct oxidation of DCFH-DA by illumination at 490 nm, intracellular levels of ROS were analysed within 3 s after illumination using a Quanticell 700 system (Applied Imaging).

Glutathione (GSH) measurement

Total glutathione (GSH plus GSSG) was determined by photometric determination of 5-thio-2-nitrobenzoate, formed from 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) at a 405 nm, according to Akerboom & Sies (1981). The assay mixture was 18 mM Tris-HCl buffer (pH 7.4) containing 0.47 mg ml^{-1} BSA, 0.007% Tween 20, 0.7 mM glucose 6-phosphate, 21.5 mM NADP, 1.4 U ml^{-1} yeast glucose 6-phosphate dehydrogenase, 0.61 mM DTNB, 0.26 U ml^{-1} glutathione reductase and 25 μl cell lysates.

Determination of catalase and superoxide dismutase (SOD) activities

Catalase activity was measured according to a modified method of Beer *et al*. (Baudhuin *et al*., 1964). The assay mixture contained 0.01 M phosphate buffer, pH 7.0 and

0.015 M hydrogen peroxide in a final volume of 1 ml and 50 μg protein of cell lysates. Changes in the optical density at 240 nm were spectrophotometrically monitored. Catalase activity was expressed as units mg^{-1} protein. One unit of enzyme activity was defined as the amount of the enzyme which decreases 1 μmol of the hydrogen peroxide per min under defined conditions. SOD activity was measured using the xanthine/xanthine oxidase and cytochrome *c* reduction assay described by McCord & Fridovich (1969). The assay mixture contained 5 μmol xanthine in 0.001 N NaOH, 2 μmol cytochrome *c* in 50 mM phosphate buffer, pH 7.8 and 0.1 mM EDTA, 0.2 U xanthine oxidase in 0.1 mM EDTA and 20 μl of cell lysates. SOD activity was expressed as units mg^{-1} protein. One unit of enzyme activity was defined as the amount of the enzyme which reduces 50% of cytochrome *c* reduction by superoxide induced by xanthine/xanthine oxidase system under defined conditions.

Statistical analysis

Statistical analysis was performed with Student's *t*-test. A *P* value of 0.05 was selected as the limit of statistical significance.

Results

FDP attenuates UVB-induced PGE_2 production and COX activity

HaCaT keratinocytes exposed to UVB irradiation dose of 30 mJ cm^{-2} showed a significant induction of PGE_2 production in a time dependent manner up to 24 h (the accumulated levels of PGE_2 were 40.72 ± 1.48, 60.86 ± 1.19, 255.4 ± 3.38, 324.4 ± 5.35 and 378.7 ± 16.74 ng mg $protein^{-1}$ at 0, 3, 6, 12 and 24 h after irradiation, respectively). Thus, levels of PGE_2 and COX-2 activity were measured 24 h after irradiation in the following experiments. FDP reduced the accumulated levels of PGE_2 in the media in a concentration 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. NS-398 was dissolved in ethanol. Final concentration of ethanol in media was 0.1% (v v^{-1}). Vehicle alone did not affect UV-induced PGE_2 production (20.85 ± 1.28 and 19.91 ± 2.24 ng ml^{-1} for control and vehicle, respectively). Total cellular COX activity was significantly increased when measured 24 h post irradiation. This increase in UVB-induced COX activity was significantly attenuated by FDP in a concentration dependent manner (Figure 2). NAC also mitigated the induction of total cellular COX activity by UVB irradiation.

FDP attenuates UVB-induced COX-2 expression

A single UVB exposure (30 mJ cm^{-2}) induced COX-2 gene expression in HaCaT cells. The maximal induction of COX-2 mRNA was observed between 3 and 6 h post irradiation (data not shown). UVB irradiation stimulated the expression of COX-2 mRNA to 3.5 fold of the control level in 3 h. This result is consistent with a previous report (Isoherranen *et al*., 1999). The increase in COX-2 expression stimulated by UVB was markedly reduced by FDP to 1.7 (20 mM) and 2.1 fold (10 mM), respectively (Figure 3). The antioxidant NAC (20 mM) suppressed UVB-induced COX-2 mRNA expression significantly (1.6 fold of control level).

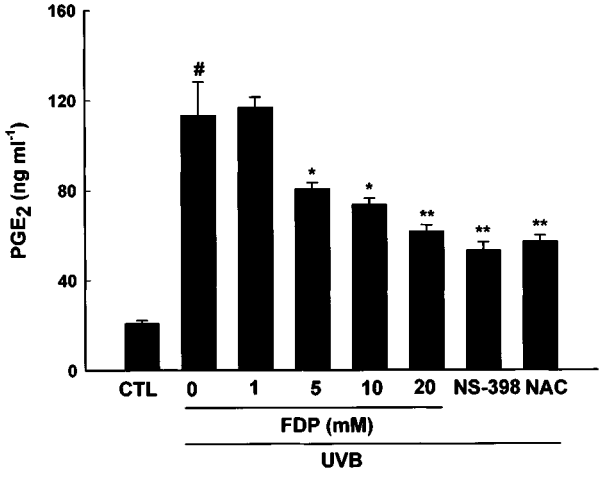

Figure 1 FDP attenuates UVB-induces PGE_2 production. HaCaT keratinocytes that had been pretreated with aspirin (100 μM) for 30 min were exposed to UV irradiation (30 mJ cm^{-2}). After the UVB exposure, cells were incubated with FDP, NS-398 (1 μM) or NAC (20 mM) in fresh media containing 10% FCS for 24 h and the accumulated levels of PGE_2 in the media were determined. The results are expressed as mean $\pm$ s.e.mean in three different experiments. UVB-irradiation significantly increased PGE_2 production compared with unirradiated control ($\#P<0.01$). A significant difference in PGE_2 concentrations relative to UVB control is indicated with $*P<0.05$ or $**P<0.01$.

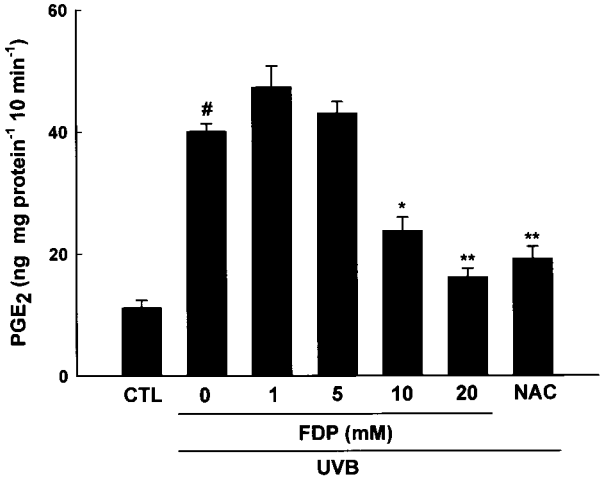

Figure 2 FDP attenuates UVB-induced COX activity. HaCaT keratinocytes that had been pretreated with aspirin (100 μM for 30 min were exposed to UV irradiation (30 mJ cm^{-2}). After the UVB exposure, cells were incubated with FDP or NAC (20 mM) in fresh culture media containing 10% FCS for 24 h. COX activity was determined as described in Methods. The results are expressed as mean $\pm$ s.e.mean in three different experiments. UVB-irradiation significantly increased COX activity compared with unirradiated control ($\#P<0.01$). A significant difference in COX activity relative to UVB control is indicated with $*P<0.05$ or $**P<0.01$.

FDP attenuates accumulation of ROS and preserves cellular total glutathione level and catalase activity after UVB irradiation

In order to explore the relevant cellular events that may be involved in the regulation of COX-2 by FDP, intracellular levels of reactive oxygen species (ROS) were analysed using DCFH-DA, a redox sensitive dye. The time course for the levels of ROS in HaCaT keratinocytes afer UVB irradiation showed that the increase of cellular ROS level was observed from 1 h post irradiation and maximum level was reached in 3 h (Figure 4a). UVB-induced ROS accumulation was attenuated to control level by FDP (10 mM) at all time points of measurement (Figure 4a). The increase in [ROS]i was significantly reduced in the presence of FDP in a concentration dependent manner measured at 3 h after irradiation (Figure 4b). FDP did not show direct radical scavenging effect in the experiment using 1,1-diphenyl-2-picrylhydrazil (DPPH) (data not shown). On the other hand, FDP preserved the cellular antioxidant capacity such as catalase and glutathione which were significantly reduced after UVB irradiation (Figure 5). UVB-irradiation reduced catalase activity to $86\pm4\%$ of control, which was reversed by FDP. Cellular glutathione content was also reduced to $83\pm7\%$ of control after irradiation, and FDP restored it to $101\pm6\%$. The baseline levels of catalase and glutathione were significantly increased in HaCaT cells treated with FDP ($125\pm4\%$, $P<0.05$ vs control; $138\pm11\%$, $P<0.05$ vs

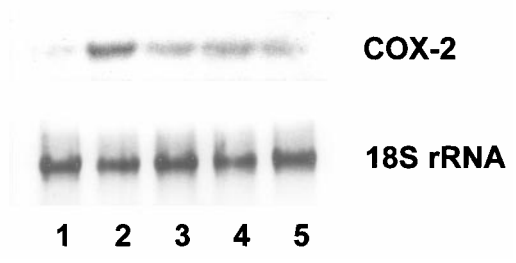

Figure 3 FDP attenuates UVB-induced COX-2 gene expression. HaCaT keratinocytes that had been pretreated with aspirin (100 μM) for 30 min were exposed to UV irradiation (30 mJ cm^{-2}). After the UVB exposure, cells were incubated with FDP or NAC (20 mM) in fresh culture media containing 10% FCS. Total RNA was extracted 3 h later, and 20 μg of total RNA was hybridized with COX-2 cDNA probe. Lane 1: unirradiated control; Lane 2: UVB control; Lane 3: UVB + FDP (20 mM); Lane 4: UVB + FDP (10 mM); Lane 5: UVB + NAC (20 mM).

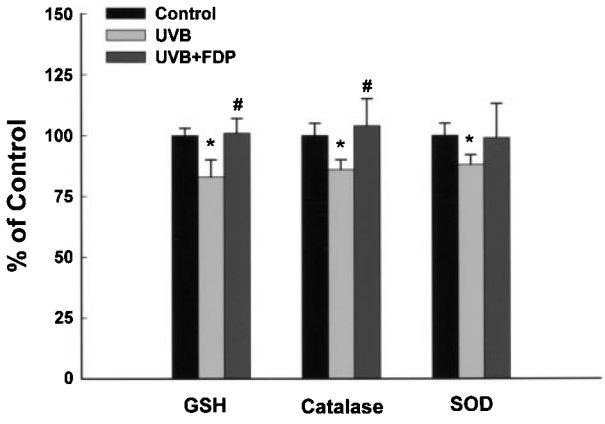

Figure 5 FDP preserves the cellular antioxidant capacity. HaCaT keratinocytes that had been exposed to UV irradiation (30 mJ cm^{-2}) were incubated with F1,6DP (10 mM) in fresh culture media containing 10% FCS for 24 h. The results are expressed as mean ± s.e.mean in four different experiments. UVB-irradiation significantly decreased the total glutathione level and activities of catalase and SOD compared with unirradiated control ($*P<0.05$). A significant difference relative to UVB control is indicated with $\#P<0.05$.

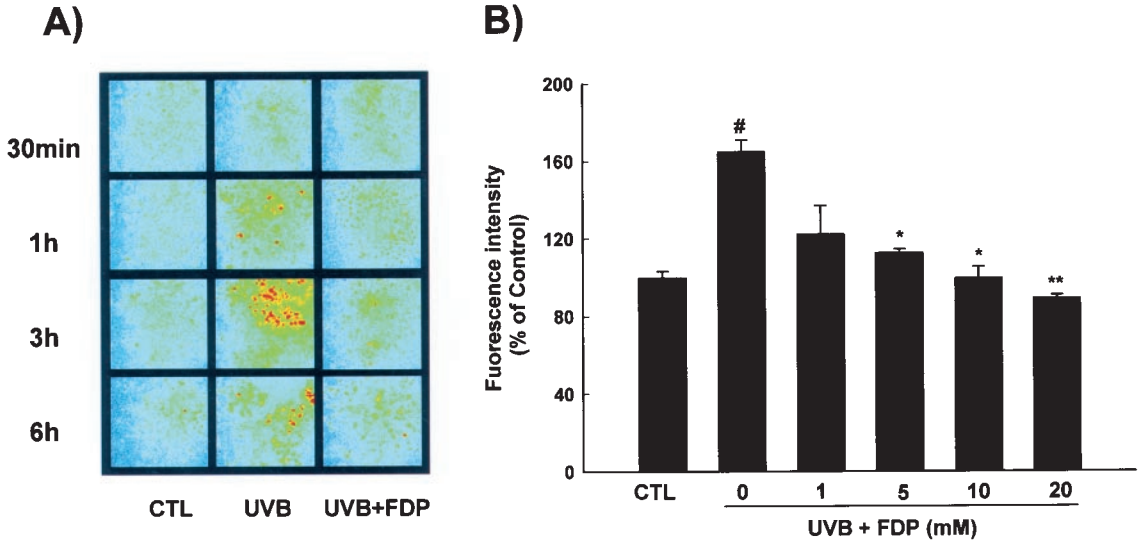

Figure 4 FDP attenuates the intracellular levels of UVB-induced reactive oxygen species (ROS). (A) Maximum level of cellular ROS was reached in 3 h after UVB-irradiation. UVB-induced ROS accumulation was reduced to control level by FDP (10 mM). (B) FDP attenuates the cellular accumulation of ROS measured at 3 h after irradiation. The results are expressed as mean ± s.e.mean in four different experiments. UVB-irradiation significantly increased ROS production compared with unirradiated control ($\#P<0.01$). A significant difference relative to UVB control is indicated with $*P<0.05$ or $**P<0.01$.

control, respectively). FDP failed to alter the cellular levels of superoxide dismutase (SOD) whether or not cells were irradiated.

Discussion

The UV-induced erythema leading to vasodilation and oedema correlates with the activation of inflammatory mechanisms including prostaglandin pathways. Previous studies have suggested that the enhanced COX-2 expression may be an important component in UVB-induced prostaglandin production and inflammatory reaction (Isoherranen *et al*., 1999; Wilgus *et al*., 2000). In the present study, we also observed that a physiological dose of UVB irradiation caused a significant induction of COX-2 expression in HaCaT keratinocytes. A single UVB exposure significantly enhanced COX activity and, thereby, the increase of prostaglandin synthesis. Together with these results, the significant inhibition of prostaglandin production by NS-398, a COX-2 selective inhibitor, further supports the role of COX-2 in UVB-induced prostaglandin synthesis.

The present study showed that FDP inhibited the induction of total cellular COX activity and abolished COX-2 expression in HaCaT keratinocytes exposed to UVB. FDP has been reported to ameliorate inflammation. Hepatitis-like injury in rats was prevented by repeated dosings of FDP (350 mg kg^{-1}, i.p. $\times 3$) at 24 h after galactosamine injection (Markov *et al*., 1991). FDP (0.5–2 g kg^{-1}, i.p. and p.o.) suppressed the paw oedema of rats at 1–5 h after local carageenan injection (Planas *et al*., 1993). FDP (100 mg kg^{-1}, i.p.) enhanced the suppression of ischaemic and histamine oedema by dexamethasone in oxyradicals and NO-dependent manner (Oyanagui, 1998). These effects of FDP have been explained by the maintenance of ATP level to stabilize cell membrane or to support ATP-dependent glucocorticoid receptor recycling or by the action to inhibit the generation of oxygen free radicals and attachment to the blood vessels of neutrophils. However, its anti-inflammatory effects in connection with COX-2 expression and prostaglandin synthesis have not been studied. Thus, our results add another possible mechanism to the anti-inflammatory action of FDP.

Increased COX-2 expression is also known to be linked to tumorigenesis. COX-2 expression is upregulated in colorectal and gastric carcinomas (Rao *et al*., 1995; Dubois *et al*., 1998) and overexpression of COX-2 produces a carcinogenic phenotype in rat intestinal epithelial cells (Thujii & Dubois, 1995). Furthermore, knockout mutation of COX-2 gene reduces the risk for intestinal polyposis in mice (Oshima *et al*., 1996). Recently it was also shown that the number of UVB-induced murine skin tumors is reduced by the selective COX-2 inhibitor, celecoxib (Pentland *et al*., 1999). Therefore, continually increased COX-2 expression after UV exposure may have an important role in UV-induced carcinogenesis. In this regard, it would be worth examining whether FDP could reverse UVB-induced skin tumorigenesis.

Recent studies have suggested that reactive oxygen intermediates contribute to the signalling events leading to gene expression after UV irradiation (Garmyn & Degreef, 1997; Tyrrell, 1996). In our study, it was observed that NAC reduced the prostaglandin production and the induction of the total cellular COX activity by UVB irradiation. NAC also mitigated the induction of UVB-induced COX-2 mRNA in HaCaT keratinocytes after UV-exposure. These results coincide with the previous reports that oxidant components play an important role in COX-2 induction after UV irradiation (Isoherranen *et al*., 1999; Soriani *et al*., 1999). In this context, intracellular ROS levels were analysed to explore the relevant cellular events that may be involved in the regulation of COX-2 by FDP. It was reported that FDP completely inhibited oxyradical generation by stimulated canine and human neutrophils although the precise mechanism was not known (Sun *et al*., 1990). In this study it was observed that FDP significantly reduced the generation of ROS after UVB irradiation and this was not due to direct radical scavenging activity. It has been suggested that FDP could be a substrate for or stimulator of anaerobic ATP production (Bickler & Buck, 1996). It was also shown that FDP might upregulate the pentose phosphate pathway (PPP), possibly by inhibiting phosphofructokinase (Kelleher *et al*., 1995; 1996; Espanol *et al*., 1999). It is well documented that the pentose phosphate pathway plays an important role in the cellular redox regulation, by providing NADPH which is the principal intracellular reductant and a critical modulator of the redox potential in all cell types (Ben-Yoseph *et al*., 1996). NADPH is used for the regeneration of glutathione and also required for the formation of active catalase tetramers (Salvemini *et al*., 1999). Our data showed that FDP preserved cellular catalase activity and glutathione levels (Figure 5) and increased the activity of glucose-6-phosphate dehydrogenase, the rate limiting enzyme of PPP, in keratinocytes exposed to UVB (data not shown). Thus, it is highly plausible that FDP reduces ROS generation *via* activation of PPP.

FDP is reported to have cytoprotective effects against ischaemia and postischaemic reperfusion injury in various tissues, presumably by augmenting anaerobic carbohydrate metabolism (Farias *et al*., 1990; Sola *et al*., 1996; Takeuchi *et al*., 1998). In this study, it was also observed that FDP could block the LDH release from HaCaT keratinocytes exposed to UVB (data not shown). Because keratinocytes are major epidermal cells injured directly by UV light, it would be of interest to test the potential application of FDP as a protective agent against UVB-induced skin damage. Together with its anti-inflammatory effect, this possibility is under investigation *in vivo*.

There is no information on endogenous level of FDP in skin. It is reported that the calculated total FDP concentrations in aerobic and ischaemic hearts are 90 and 250 μM, respectively (Hardin *et al*., 2001). Thus, on the assumption that skin and heart contain the same levels of FDP, the levels being used exogenously in this study is more than 10 times of endogenous level. Because FDP is a highly charged molecule at physiological pH, it is generally not expected to readily cross the cell membrane. However, it was reported that metabolism of exogenous fructose was undetectable or if any, only about 10% of that found with FDP in vascular smooth muscle and heart, providing evidence that FDP enters cells rather than being converted to fructose (Hardin & Roberts, 1995; Tavazzi *et al*., 1992). It has been demonstrated that FDP is capable of crossing artificial lipid bilayers in a concentration dependent manner (Ehringer *et al*., 2000). Furthermore, a recent report showed FDP could be transported into the cell *via* a dicarboxylate transport system

(Hardin *et al*., 2001). These results strongly suggest that FDP has an intracellular site of action. Whether or not this speculation is true, our data add the possible usefulness for UVB-induced skin disorder to the list of the beneficial effects of FDP such as the prevention of endotoxic shock (Markov *et al*., 1981) and ischaemic injury in a wide variety of tissues including brain (Sola *et al*., 1996), heart (Takeuchi *et al*., 1998, kidney (Didlake *et al*., 1989) and intestine (Sun *et al*., 1990) and amelioration of traumatic (Markov *et al*., 1983) and inflammatory disease (Planas *et al*., 1993; Oyanagui, 1998).

In summary, our data hitherto obtained show that FDP could attenuate UVB-induced COX-2 expression, thereby reducing the prostaglandin production in HaCaT keratinocytes and this could be ascribed, at least in part, to the diminution of intracellular oxidative stress although the nature of its effects on generation of reactive oxygen species remains to be elaborated.

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References

AKERBOOM, T.P.M. & SIES, H. (1981). Assay of glutathione, glutathione disulfide, and glutathione mixed disulfides in biological samples. In *Methods in Enzymology 77: Detoxication and Drug Metabolism: Conjugation and Related systems*. ed. Jakoby W.B. pp. 373 – 382. New York: Academic Press.

ATHAR, M., AN, K.P., MOREL, K.D., KIM, A.L., ASZTERBAUM, M., LONGLEY, J., EPSTEIN JR., E.H. & BICKERS, D.R. (2001). Ultraviolet B (UVB)-induced COX-2 expression in murine skin: an immunohistochemical study. *Biochem. Biophys. Res. Commun.,* **280,** 1042 – 1047.

BAUDHUIN, P., BEAUFAY, H., RAHMAN-LI, Y., SELLINGER, O.Z., WATTIAUX, R., JACQUES, P. & DE DUVE, C. (1964). Intracellular distribution of monoamine oxidase, aspartate aminotransferase, alanine aminotransferase, D-amino acid oxidase and catalase in rat liver tissue. *Biochem. J.,* **92,** 179 – 184.

BEN-YOSEPH, O., BOXER, P. & ROSS, B. (1996). Assessment of the role of the glutathione and pentose phosphate pathways in the protection of primary cerebrocortical cultures from oxidative stress. *J. Neurochem.,* **66,** 2329 – 2337.

BICKLER, P.E. & BUCK, L.T. (1996). Effects of fructose-1,6-bisphosphate on glutamate release and ATP loss from rat brain slices during hypoxia. *J. Neurochem.,* **67,** 1463 – 1468.

BOUKAMP, P., PETRUSSEVSKA, R.T., BREITKREUTZ, D., HORNUNG, J., MARKHAM, A. & FUSENIG, N.E. (1988). Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J. Cell Biol.,* **106,** 761 – 771.

CHEN, X., GRESHAM, A., MORRISON, A. & PENTLAND, A.P. (1996). Oxidative stress mediates synthesis of cytosolic phospholipase A_2 after UVB injury. *Biochim. Biophys. Acta,* **1299,** 23 – 33.

DIDLAKE, R.H., KIRCHNER, K.A., LEWIN, J., BOWER, J.D. & MARKOV, A.K. (1989). Attenuation of ischemic renal injury with fructose-1,6-diphosphate. *J. Surg. Res.,* **47,** 220 – 226.

DUBOIS, R.N., ABRAMSON, S.B., CROFFORD, L., GUPTA, R.A., SIMON, L.S., VAN DE PUTTE, L.B. & LIPSKY, P.E. (1998). Cyclooxygenase in biology and disease. *FASEB J.,* **12,** 1063 – 1073.

EDDE, L., ZHOU, Z., EATON, J.W. & SHERMAN, M.P. (1998). Induction of nitric oxide synthase in macrophages: inhibition by fructose-1,6-diphosphate. *Biochem. Biophys. Res. Commun.,* **243,** 683 – 687.

EHRINGER, W.D., NIU, W., CHIANG, B., WANG, O.L., GORDON, L. & CHIEN, S. (2000). Membrane permeability of fructose-1,6-diphosphate in lipid vesicles and endothelial cells. *Mol. Cell. Biochem.,* **210,** 35 – 45.

ESPANOL, M.T., LITT, L., HASEGAWA, K., CHANG, L., MACDONALD, J.M., GREGORY, G., JAMES, T.L. & CHAN, P.H. (1999). Fructose-1,6-bisphosphate preserves adenosine triphosphate but not intracellular pH during hypoxia in respiring neonatal rat brain slices. *Anesthesiology,* **88,** 461 – 472.

FARIAS, L.A., SMITH, E.E. & MARKOV, A.K. (1990). Prevention of ischemic-hypoxic brain injury and death in rabbits with fructose-1,6-diphosphate. *Stroke,* **21,** 606 – 613.

FARR, P.M. & DIFFEY, B.L. (1986). A quantitative study of the effect of topical indomethacin on cutaneous erythema induced by UVB and UVC radiation. *Br. J. Dermatol.,* **115,** 453 – 466.

FU, J.Y., MASFERRER, J.L., SEIBERT, K., RAZ, A. & NEEDLEMAN, P. (1990). The induction and suppression of prostaglandin H_2 synthase (cyclooxygenase) in human monocytes. *J. Biol. Chem.,* **265,** 16737 – 16740.

GARMYN, M. & DEGREEF, H. (1997). Suppression of UVB-induced c-fos and c-jun expression in human keratinocytes by N-acetylcysteine. *J. Photochem. Photobiol. B.,* **37,** 125 – 130.

GREAVES, M.W. & SONDERGAARD, J. (1970). Pharmacological agents released in ultraviolet inflammation studied by continuous skin perfusion. *J. Invest. Dermatol.,* **54,** 365 – 367.

GRESHAM, A., MASFERRER, J., CHEN, X., LEAL-KHOURI, S. & PENTLAND, A.P. (1996). Increased synthesis of high molecular weight cPLA2 mediates early UV-induced PGE_2 in human skin. *Am. J. Physiol.,* **270,** C1037 – C1050.

HARDIN, C.D., LAZZARINO, G., TAAZZI, B., DI PIERRO, D., ROBERTS, T.M., GIARDINA, B. & ROVETTO, M.J. (2001). Myocardial metabolism of exogenous FDP is consistent with transport by a dicarboxylate transporter. *Am. J. Physiol. Heart Circ. Physiol.,* **281,** H2654 – H2660.

HARDIN, C.D. & ROBERTS, T.M. (1995). Compartmentation of glucose and fructose 1,6-bisphosphate metabolism in vascular smooth muscle. *Biochemistry,* **34,** 1323 – 1331.

HRUZA, L. & PENTLAND, A.P. (1993). Mechanisms of UV-induced inflammation. *J. Invest. Dermatol.,* **100,** 35S – 41S.

IBBOTSON, S.H., DIFFEY, B.L. & FARR, P.M. (1996). The effect of topical indomethacin on ultraviolet-radiation-induced erythema. *Br. J. Dermatol.,* **135,** 523 – 527.

ISOHERRANEN, K., PUNNONEN, K., JANSEN, C. & UOTILA, P. (1999). Ultraviolet irradiation induces cyclooxygenase-2 expression in keratinocytes. *Br. J. Dermatol.,* **140,** 1017 – 1022.

KANG-ROTONDO, C.H., MILLER, C.C., MORRISON, A.R. & PENTLAND, A.P. (1993). Enhanced keratinocyte prostaglandin synthesis after UV injury is due to increased phospholipase activity. *Am. J. Physiol.,* **264,** C396 – C401.

KELLEHER, J.A., CHAN, P.H., CHAN, T.Y.Y. & GREGORY, G.A. (1995). Energy metabolism in hypoxic astrocytes: protective mechanism of fructose-1,6-bisphosphate. *Neurochem. Res.,* **20,** 785 – 792.

KELLEHER, J.A., CHAN, T.Y.Y., CHAN, P.H. & GREGORY, G.A. (1996). Protection of astrocytes by fructose 1,6-bisphosphate and citrate ameliorates neuronal injury under hypoxic conditions. *Brain Res.,* **726,** 167 – 173.

MARKOV, A.K., FARIAS, L.A., BENNETT, W.S., SUBRAMONY, C. & MIHAS, A.A. (1991). Prevention of galactosamine-induced hepatotoxicity in rats with fructose-1,6-diphosphate. *Pharmacology,* **43,** 310 – 317.

MARKOV, A.K., OGLETHORPE, N.C., GRILLIS, M., NEELY, W.A. & HELLEMS, H.K. (1983). Therapeutic action of fructose-1,6-diphosphate in traumatic shock. *World J. Surg.,* **7,** 430 – 436.

MARKOV, A.K., TURNER, M.D., OGLETHORPE, N.C., NEELY, W.A. & HELLEMS, H.K. (1981). Fructose-1,6-diphosphate: an agent for treatment of experimental endotoxin shock. *Surgery,* **90,** 482 – 488.

MCCORD, J. & FRIDOVICH, I. (1969). Superoxide dismutase; An enzymatic function for erythrocuprein. *J. Biol. Chem.,* **244,** 6049 – 6055.

OSHIMA, M., DINCHUK, J.E., KARGMAN, S.L., OSHIMA, H., HANCOCK, B., KWONG, E., TRZASKOS, J.M., EVANS, J.F. & TAKETO, M.M. (1996). Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell,* **87,** 803 – 809.

OYANAGUI, Y. (1998). Fructose-1,6-diphosphate enhanced oxyradicals and nitric oxide-dependent suppressions by dexamethasone of ischemic and histamine paw edema of mice. *Life Sci.,* **62,** 241 – 249.

PENTLAND, A.P., SCHOGGINS, J.W., SCOTT, G.A., KAHN, K.N.M. & HAN, R. (1999). Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. *Carcinogenesis,* **20,** 1939 – 1944.

PLANAS, M.E., SANCHEZ, S., GONGALEZ, P., RODRIGUES DE OLIVEIRA, J. & BARTRONS, R. (1993). Protective effect of fructose 1,6-bisphosphate against carrageenan-induced inflammation. *Eur. J. Pharmacol.,* **237,** 251 – 255.

RAO, C.V., RIVENSON, A., SIMI, B., ZANG, E., KELLOFF, G., STEELE, V. & REDDY, B.S. (1995). Chemoprevention of colon carcinogenesis by sulindac, a non-steroidal anti-inflammatory agent. *Cancer Res.,* **55,** 1464 – 1472.

SALVEMINI, F., FRANZE, A., IERVOLINO, A., FILOSA, S., SALZANO, S. & URSINI, M.V. (1999). Enhanced glutathione levels and oxidoresistance mediated by increased glucose-6-phosphate dehydrogenase expression. *J. Biol. Chem.,* **274,** 2750 – 2757.

SOLA, A., BERRIOS, M., SHELDON, R.A., FERREIRO, D.M. & GREGORY, G.A. (1996). Fructose 1,6-bisphosphate after hypoxic ischemic injury is protective to the neonatal rat brain. *Brain Res.,* **742,** 294 – 299.

SORIANI, M., LUSCHER, P. & TYRRELL, R.M. (1999). Direct and indirect modulation of ornithine decarboxylase and cyclooxygenase by UVB in human skin cells. *Carcinogenesis,* **20,** 727 – 732.

SUN, J., FARIAS, L.A. & MARKOV, A.K. (1990). Fructose 1,6 diphosphate prevents intestinal ischemic reperfusion injury and death in rats. *Gastroenterology,* **98,** 117 – 126.

TAKEUCHI, K., CAO-DANH, H., FRIEHS, I., GLYNN, P., D'AGOSTINO, D., SIMPLACEANU, E., MCGOWAN, F.X. & DEL NIDO, P.J. (1998). Administration of fructose-1,-6-diphosphate during early reperfusion significantly improves recovery of contractile function in the postischemic heart. *J. Thorac. Cardiovasc. Surc.,* **116,** 335 – 343.

TAVAZZI, B., STARNES, J.W., LAZZARINO, G., DI PIERRO, D., NUUTINEN, E.M. & GIARDINA, B. (1992). Exogenous fructose-1,6-bisphosphate is a metabolizable substrate for the isolated normoxic rat heart. *Basic Res. Cardiol.,* **87,** 280 – 289.

THUJII, M. & DUBOIS, R.N. (1995). Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. *Cell,* **83,** 493 – 501.

TYRRELL, R.M. (1996). Activation of mammalian gene expression by the UV component of sunlight-from models to reality. *Bioessays,* **18,** 139 – 148.

WILGUS, T.A., ROSS, M.S., PARRETT, M.L. & OBERYSZYN, T.M. (2000). Topical application of a selective cyclooxygenase inhibitor suppresses UVB mediated cutaneous inflammation. *Prostaglandins Other Lipid Mediat.,* **62,** 367 – 384.

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