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Brief Communication

UVB Irradiation Increases the Expression of Pro-inflammatory Cytokine in Jurkat T Cells Exposed to Triglycerides

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Abscopal effect is a form of secondary immune response that occurs in ionizing radiation therapy, resulting in changes in the immune response through activation of immune cells such as macrophages and T lymphocytes. UVB causes DNA damage similar to ionizing radiation and causes similar intracellular reactions, so it is often used as an alternative in research on the effects of ionizing radiation. In a previous study, we found that pro-inflammatory cytokines, including TNF-α, increased in Jurkat T cells exposed to TGs. In this study, we confirmed the effects of UVB irradiation on T lymphocytes exposed to TGs, similar to the effects of ionizing radiation. As a result, it was shown that the mRNA expression of pro-inflammatory cytokines such as IL-1β and IFN-γ in Jurkat T cells exposed to TGs increased by UVB irradiation. In addition, it was confirmed that the increase in the expression of pro-inflammatory cytokines caused by UVB was caused by the activation of iNOS protein. This is very similar to the immune response that occurs when T lymphocytes are exposed to TGs. These results suggest that activation of iNOS protein is involved in the increase in pro-inflammatory cytokines caused by UVB irradiation in T lymphocytes exposed to TGs.

Key Words: UVB irradiation, iNOS, Jurkat T cells, Pro-inflammatory cytokines, TGs

Ionizing radiation therapy is performed on approximately 50% of cancer patients, and it is reported that approximately 40% of patients are cured of their cancer with continuous management (Harrington et al., 2011). It is known that treatment with ionizing radiation primarily causes DNA damage in cancer cells, thereby killing them (Camphausen and Tofilon, 2004). Currently, the action of killing other cancer cells by stimulating the cellular immune system occurs secondarily, and this action is called the 'Abscopal effect' (Verma and Lin, 2016). This action begins when antigen presenting cells (APC) are stimulated according to the damaged associated molecular pattern (DAMP) that appears during cancer cell death. Due to this stimulation, T cells are stimulated through MHC class II related molecules, and through this, cytotoxic T cells are activated and target other cancer cells (Verma and Lin, 2016) (Demaria et al., 2004). Therefore, to produce a secondary 'Abscopal effect' during ionizing radiation treatment, the immunological activation mechanism of macrophages and T cells is essential.

Due to westernized eating habits, many modern people are oversaturated with nutrients and suffer from various metabolic diseases (Lozano et al., 2012). Hypertriglyceridemia, in which the concentration of triglycerides (TGs) in the blood increases, is one of the representative metabolic diseases

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(Bersot et al., 2006). When the concentration of TGs in the blood increases, it affects various immune cells such as macrophages and lymphocytes involved in the immune response (Aflaki et al., 2011). One of these changes is accompanied by an increase in inflammatory response or simultaneous immune dysfunction due to a decrease in the number of immune cells due to programmed cell death (PCD) of immune cells (Aronis et al., 2008) (Lim et al., 2017). Therefore, when patients with hypertriglyceridemia receive ionizing radiation therapy for cancer treatment, etc., there is a high possibility that the immune response by immune cells will be different from that of general patients with normal blood TGs concentration.

In a previous study, it was confirmed that decrease of cell viability of T lymphocytes exposed to high concentration of TGs. In this process, it was confirmed that the mRNA expression level of pro-inflammatory cytokines, including TNF-α, increased in T lymphocytes exposed to TGs. Therefore, in this study, a T lymphocyte cell line model exposed to TGs was used for research. A similar effect was achieved using UVB instead of ionizing radiation. Ultraviolet (UV) irradiation is a cause of cellular damage similar to x-ray, γ-ray irradiation, genotoxic chemicals, virus and mechanical stress and leads to DNA damage (Ishida and Sakaguchi, 2007) (Verma and Lin, 2016) (Friedman, 2002). In this study, we used Jurkat T cells, a T lymphocyte cell line exposed to high concentrations of TGs, to study changes in the number of cells that appear upon exposure to UVB and effect on the expression level of pro-inflammatory cytokines.

TG emulsion (Lipofundin® MCT/LCT 20%) was purchased from B. Braun Melsungen AG (Melsungen, Germany). Lipofundin® MCT/LCT 20% was used to deliver TG into cells in previous studies. The composition of Lipofundin® MCT/LCT 20% was as follows: 100 g/L medium chain triglyceride, soybean oil, glycerol, egg lecithin, all-rac-α-tocopherol, sodium oleate and water. Hereafter, Lipofundin® MCT/LCT 20% will be referred to as TG for convenience. TRIzol® for RNA isolation was obtained from Invitrogen (Carlsbad, CA, USA). iNOS inhibitor 1,400 W was obtained from Sigma-Aldrich (St. Louis, MO, USA). All inhibitors were dissolved as stock solutions in DMSO and stored at -20℃ prior to usage.

The Jurkat acute T lymphocyte leukemia cell line (ATCC, Manassas, VA, USA) was grown in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 1% MEM non-essential amino acid solution, 1 mM HEPES, 100 μM sodium pyruvate and 1% penicillin-streptomycin and maintained at 37℃ in a humidified atmosphere with 5% CO_2. Jurkat cells were seeded in 6-well plates at a density of 2.5×10^5 cells/well for 24 h. Thereafter, Jurkat cells were incubated with TG and/or chemical inhibitors for 48 h.

Jurkat cells were seeded in 6-well plates at a density of 2.5×10^5 cells/well for 24 h. After 24 h, cells were irradiated with UV light (UVB, 312 nm) by UV crosslinker XL-1000 (Spectroline, Melville, NY, USA). The cells were cultured for further analysis.

Trypan blue stain solution (10 μL) was mixed with the cell suspension (10 μL) to enumerate viable cells, and unstained viable cells were counted with a hemocytometer (Marienfeld, Lauda-Königshofen, Germany). Each experiment was performed at least three times, and the results are expressed as the mean ± SEM for each group.

Total RNA was isolated from Jurkat T lymphocytes using Trizol® reagent according to the manufacturer's instructions. cDNA was synthesized by reverse transcription with 2 μg total RNA, 0.25 μg of random hexamer (Invitrogen) and 200 unit of Moloney murine leukemia virus reverse transcriptase (MMLV-RT; Invitrogen) for 10 min at 25℃, 50 min at 37℃ and 15 min at 70℃. cDNA was PCR amplified using Prime *Taq* premix PCR kit (Genet Bio, Chungnam, Korea) for 25~50 cycles using specific primers. Primer sequences are as follows: TNF-α; 5'- AGC CCA TGT TGT AGC AAA CC -3' (forward), 5'- CTG AGT CGG TCA CCC TTC TC -3' (reverse), IL-1β; 5'- AGC CAT GGC AGA AGT ACC T -3' (forward), 5'- CAG CTC TCT TTA GGA AGA CA -3' (reverse), IFN-γ; 5'- ACC GAA TAA TTA GTC AGC TT -3' (forward), 5'- AGT TAT ATC TTG GCT TTT CA -3' (reverse). GAPDH was used as an internal control. PCR products were electrophoresed on 2% (w/v) agarose gels containing 0.5 μg/mL ethidium bromide and the product size determined by comparison to 100 bp DNA ladder marker (Intron, Gyeonggi, Korea). Gel images were taken using Gel Doc™ XR+ system (Bio-Rad, Hercules,

CA, USA). The PCR product band intensity was measured and normalized against GAPDH using Image Lab™ software (version 4.1, Bio-Rad).

Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). The *P*-values were calculated using Student's *t*-test. Data are presented as mean ± standard error of the mean (SEM). Each experiment was conducted three times, and data were pooled for analysis. The differences were considered statistically significant at $^{*}P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.001$.

In a previous study, it was confirmed that the cell viability of T lymphocytes exposed to TGs decreased in a time-dependent and dose-dependent manner. Therefore, we confirmed whether UVB affects the decrease in the cell viability of T lymphocytes expose to TGs. As a result, it was confirmed that when only 1 mg/mL of TGs was treated, the cell viability of Jurkat T lymphocyte cells decreased statistically significantly after 24 h (Fig. 1A). This is a similar result to previous research results. The effect of UVB irradiation was confirmed, there was no significant difference in cell survival rate within 24 h. However, after 48 h of UVB irradiation at 200 J/m^2, it was confirmed that the cell viability was significantly reduced (Fig. 1B). Referring to this result, T lymphocytes treated with TGs were irradiated with 100 J/m^2 UVB to confirm the cell viability. As a result, it was confirmed that there was no significant change in cell viability caused by UVB even after 48 h (Fig. 1C). All subsequent experiments were conducted with reference to the experimental conditions.

Previously, we confirmed that the expression of pro-inflammatory cytokines, including TNF-α, increased in T lymphocytes exposed to TGs. To confirm the effect of UVB on the expression of pro-inflammatory cytokines, T lymphocyte cells treated with TGs were irradiated with UVB and the expression level was confirmed. In the case of TNF-α, it was confirmed that there was no significant change in expression level even after UVB irradiation (Fig. 2A). However, in the case of IL-1β, different results were shown. It was confirmed that the expression level of IL-1β, which was not affected by TGs, was increased by UVB irradiation (Fig. 2B). IFN-γ also showed similar results (Fig. 2C). These results demonstrate that UVB irradiation induces the mRNA

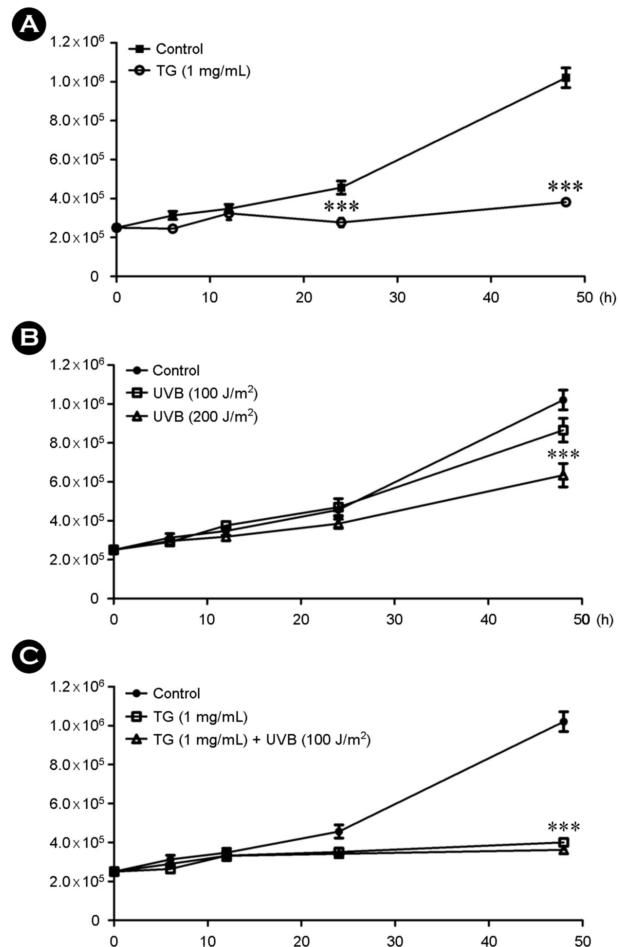

Fig. 1. UVB has no significant effect on cell viability of Jurkat T cells. (A) Jurkat T cells were treated with TG (1.0 mg/mL) for the indicated times (0, 6, 12, 24, or 48 h). **(B)** Jurkat T cells were irradiated with indicated doses (0, 100, or 200 J/m^2) of UVB for indicated times (0, 6, 12, 24, or 48 h). **(C)** Jurkat T cells were treated with TG (1.0 mg/mL) and irradiated with UVB (100 J/m^2) for indicated times (0, 6, 12, 24, or 48 h). Viable cells were enumerated by the trypan blue dye exclusion assay. All data are expressed as the mean ± SEM of three independent experiments. *P*-values were determined with two-way ANOVA followed by Bonferroni post-hoc test. $^{***}P < 0.001$.

expression of pro-inflammatory cytokines in T lymphocytes independently of the exposed TGs.

According to Aronis et al., the response within immune cells due to exposure to TGs is related to reactive oxygen species (ROS) generation (Aronis et al., 2005). And in a previous study, our research confirmed that inducible nitric oxide synthase (iNOS) protein, which is related to ROS production, is involved in reducing cell viability and increasing pro-inflammatory cytokine expression. Therefore,

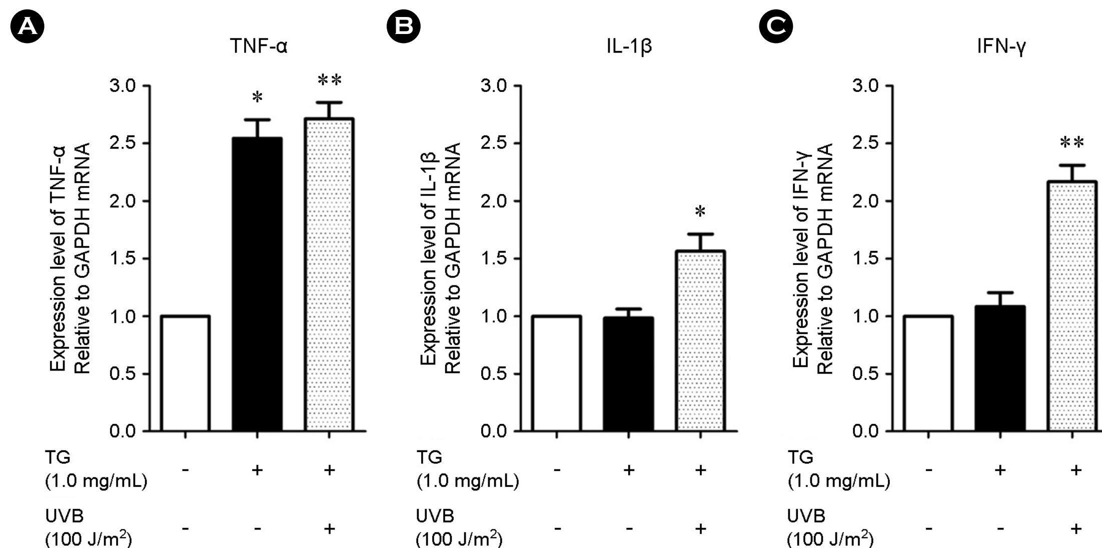

Fig. 2. UVB irradiation increases the expression of pro-inflammatory cytokines in Jurkat T cells exposed to TGs. Jurkat T cells were treated with TG (1.0 mg/mL) and irradiated with UVB (100 J/m^2) for 24 h. **(A)** The mRNA level of TNF-α was analyzed using RT-PCR. **(B)** The mRNA level of IL-1β was analyzed using RT-PCR. **(C)** The mRNA level of INF-γ was analyzed using RT-PCR. GAPDH was used as internal control. Data are expressed as the mean ± SD and are presented as the expression levels of mRNA relative to GAPDH mRNA. The expression level of mRNA relative to GAPDH in Jurkat T cells without treatment TG and UVB was set as 1.0. Data are from three independent experiments. *P*-values were determined with Student's *t*-test. *$P < 0.05$, **$P < 0.01$.

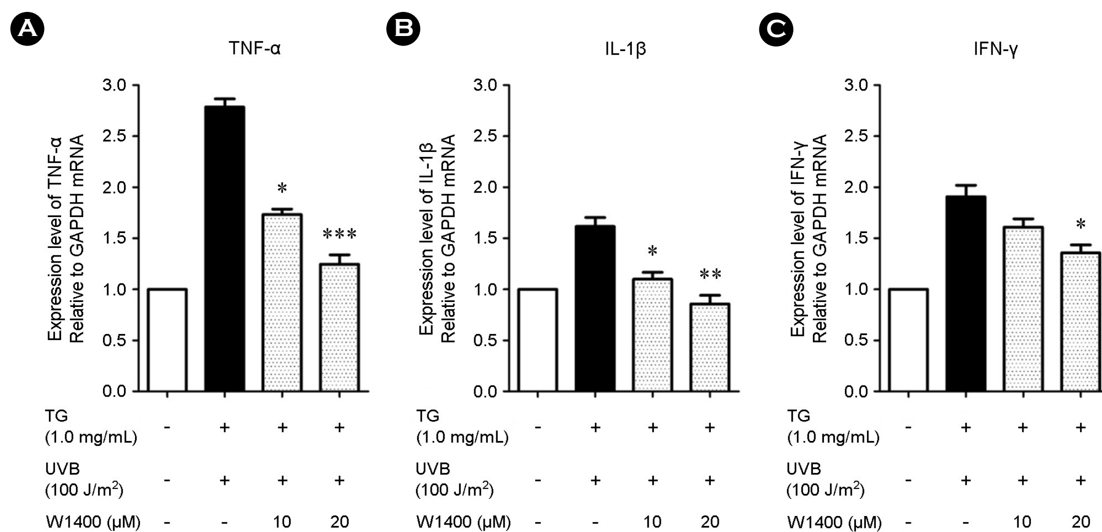

Fig. 3. iNOS mediates UVB-induced increased expression of pro-inflammatory cytokines in Jurkat T cells exposed to TGs. Jurkat T cells were treated with TG (1.0 mg/mL) and irradiated with UVB (100 J/m^2) in the absence or presence of the iNOS inhibitor 1,400 W (0, 10, or 20 μM) for 24 h. **(A)** The mRNA level of TNF-α was analyzed using RT-PCR. **(B)** The mRNA level of IL-1β was analyzed using RT-PCR. **(C)** The mRNA level of INF-γ was analyzed using RT-PCR. GAPDH was used as internal control. Data are expressed as the mean ± SD and are presented as the expression levels of mRNA relative to GAPDH mRNA. The expression level of mRNA relative to GAPDH in Jurkat T cells without treatment TG and UVB was set as 1.0. Data are from three independent experiments. *P*-values were determined with Student's *t*-test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

to confirm the relationship between UVB irradiation and iNOS protein, an experiment was conducted using w1400, one of the iNOS protein inhibitors. As a result, it was confirmed that the pro-inflammatory cytokines increased by

simultaneous exposure to TGs and UVB irradiation were partially recovered (Fig. 3). In the case of TNF-α and IL-1β, expression levels were almost similar to those of the control group. However, in the case of IFN-γ, only about half of the increased expression level was recovered. These results suggest that iNOS is involved in increasing the expression of IL-1β and IFN-γ by UVB irradiation, in addition to increasing the expression of TNF-α by TGs. In addition, it shows the possibility that an iNOS-independent signaling is involved in the increase in IFN-γ due to UVB irradiation.

Hypertriglyceridemia is known to affect immune cells in the circulatory system and cause various inflammatory diseases in the circulatory system, including atherosclerosis (Malloy and Kane, 2001). Previously, we reported that the cell viability of T lymphocytes exposed to TGs decreases due to the programmed cell death (PCD) process. In this process, it was confirmed that the expression level of TNF-α increased due to activation of signaling via iNOS protein. In this study, we confirmed that when T lymphocytes exposed to TGs were exposed to UVB irradiation, the expression of other pro-inflammatory cytokines, IL-1β and IFN-γ, was independently increased. In addition, similar to TGs, it was confirmed that the expression of IL-1β and IFN-γ was increased by the signaling pathway via the iNOS protein.

Previous study has demonstrated that one of the effects of ionizing radiation is iNOS protein activity in circulatory immune cells such as monocytes and T lymphocytes (Formenti and Demaria, 2009) (Kadhim et al., 2013). Additionally, this effect is known to be caused by UVB, which has a similar effect to ionizing radiation. In this study, a similar increase in the expression levels of IL-1β and IFN-γ was confirmed through the activation of iNOS. However, in the case of TNF-α, which is commonly known to be involved, no significant changes were observed when UVB irradiation was performed on T lymphocytes exposed to TGs. Various studies have reported that the expression of TNF-α and IL-1β increases simultaneously in macrophages, lymphocytes, or granulocytes during UVB irradiation. Therefore, in terms of the abscopal effect, there is a possibility that T lymphocytes exposed to TGs may show differences in the TNF-α-mediated response. In addition, it is necessary to conduct additional detailed intracellular mechanism studies to confirm the effects of TGs.

This study showed that more pro-inflammatory cytokines were expressed in T lymphocytes when exposed to TGs and UVB simultaneously, compared to when exposed to TGs alone. In several other studies, it was reported that when normal human epidermal keratinocyte (NHEK) cell line was exposed to UVB, the expression level of cytokines related to inflammatory response, such as IL-1α, IL-1β, and IL-6, increased within 24 h (Ishida and Sakaguchi, 2007). Therefore, the effect of activating the immune response by UVB irradiation is likely to be stronger when T lymphocytes in the blood are exposed to high concentrations of TGs.

Various studies have described the correlation between the activation of iNOS protein in various immune cells, including T lymphocytes, and inflammatory immune responses. According to studies, UVB exposure causes a reaction accompanied by DNA damage, and this process is associated with the iNOS protein. And the generation of nitric oxide (NO) by iNOS promotes the expression of inflammatory cytokines (Aflaki et al., 2011) (Aronis et al., 2005). In this study, it was similarly confirmed that activation of iNOS protein was involved in increasing the expression of IL-1β and IFN-γ. Additionally, in a previous study, we reported that exposure to TGs also increases pro-inflammatory cytokines, including TNF-α, via the iNOS activation. Therefore, it was confirmed that both TGs and UVB commonly increase the expression level of pro-inflammatory cytokines mediated by iNOS activation. Of course, there were differences in the target cytokines, but it is highly likely that the iNOS protein plays a key role in the cell signaling pathway.

In conclusion, these studies demonstrate that activation of iNOS protein is involved in the increase in pro-inflammatory cytokines caused by UVB irradiation in T lymphocytes exposed to TGs.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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