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Triglycerides increase mRNA Expression of Pro-inflammatory Cytokines Via the iNOS in Jurkat T lymphocyte and U937 Monocyte Cell Lines

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ABSTRACT

Triglycerides (TG) are one of the triggers of chronic inflammatory lesions in the blood vessels. In the key factors in the development of inflammatory diseases, Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) contribute to the development of inflammatory lesions by recruiting other immune cells in the inflamed area or causing cell necrotic death. In this study, I investigated the effect of Jurkat T lymphocytes and U937 monocytes involved in vascular inflammation development on the expression of TNF-α and IL-1β on exposure to TGs. In Jurkat cells, mRNA expression of TNF-α is increased by exposure to TGs. However, the expression levels of TNF-α and IL-1β were increased by TGs in U937 cells. To investigate whether inducible nitric oxide synthase (iNOS) is involved in the increase of expression of TNF-α and IL-1β by TGs, treatment of W1400 (an iNOS inhibitor) resulted in recovery of expression level both TNF-α and IL-1β. Based on the present study, it was confirmed that the expression of TNF-α and IL-1β in monocytes and T lymphocytes. This increased cytokines contribute to development of vascular inflammatory lesions. In addition, iNOS is involved in the increase of TNF-α and IL-1β expression by TGs.

Key Words: triglyceride (TG), TNF-α, IL-1β, iNOS

Ⅰ. INTRODUCTION

Triglycerides (TGs), independent of cholesterol, is known to be a factor causing chronic inflammatory diseases in the blood vessels such as atherosclerosis.[1] In the Framingham Heart Study (FHS), the effects of hypertriglyceridemia on coronary artery disease (CAD), including atherosclerosis, showed that the probability of developing CAD more than doubled when the serum concentration of TGs increased by more than 250 mg/dL, Respectively.[2,3] However, the detailed functional mechanism of increased blood TG levels in the development of inflammatory lesions is not well known.

In the mechanism of most inflammatory reactions, the immune cells penetrate into the lesion and the infiltrated cells exacerbate inflammation. In most cases, T cells are first activated to secrete cytokines such as interleukin (IL)-4, IL-10 and transforming growth factor beta (TGF-β), which have anti-inflammatory effects. At the same time, T cells secrete inflammatory cytokines such as tumor necrosis factor-alpha (TNF-)α and IL-1β.[4-8] And if T cells stimulate macrophages by these factors, they cause cascade of cytokine secretion of macrophages.[9] In particular, TNF-α and IL-1β secreted by macrophages inhibit the expression of anti-inflammatory cytokines secreted by T-cells and stimulate inflammatory cells. This is a major factor in the development of chronic inflammation.[4]

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TNF-α and IL-1β are also secreted into lesions during the onset of atherosclerosis and promote the death of other cells in blood vessels.[10,11] In addition to atherosclerosis, intravascular cells are easily stimulated by external stimuli and should be considered in the treatment of disease. In radiotherapy for cancer, Ionizing radiation (such as X-rays, gamma rays, and electron beams) has been reported to activate inducible nitric oxide synthase (iNOS) in both T cells and macrophages.[12] iNOS is an enzyme that produces nitric oxide from L-arginine and can secrete to the outside of the cell, and the produced nitric oxide (NO) acts as a molecule important for cell signal transduction and regulates the immune response.[13]

Although previous studies have shown that TG is involved in the death of inflammatory cells and secretion of cytokines, there is no known correlation with iNOS. In this study, I determined the effects of triglyceride, which is involved in the development of chronic inflammatory diseases in blood vessel, on the expression of inflammatory cytokines-secreted by T cell and mononuclear cells. I then investigated the pathogenesis of atherosclerosis by TG.

Ⅱ. MATERIAL AND METHODS

1. Materials

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 Aronis et al., 2005. TRIzol® for RNA isolation was obtained from Invitrogen (Carlsbad, CA, USA). Chemical inhibitors for iNOS (W1400) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The iNOS inhibitor was dissolved as stock solutions in DMSO and stored at -20°C prior to usage.

2. Cell culture

The Jurkat T lymphocyte cell line (ATCC, Manassas, VA, USA) and U937 monocyte cell line (ATCC) were grown in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and maintained at 37°C in a humidified atmosphere with 5% CO_2. Jurkat cells and U937 cells were seeded in 6-well plates at a density of 2.5×10^5 cells/well for 24 h. Thereafter, these cells were incubated with TG and/or chemical inhibitors for 48 h.

3. RNA extraction and semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR)

Total RNA was isolated from Jurkat T lymphocytes using Trizol® reagent according to the manufacturer's instructions. Complementary DNA (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°C, 50 min at 37°C and 15 min at 70°C. 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). Glyceraldehyde 3-phosphate dehydrogenase (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

DocTM XR+ system (Bio-Rad, Hercules, CA, USA). The PCR product band intensity was measured and normalized against GAPDH using Image LabTM software (version 4.1, Bio-Rad).

4. Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA). p-values were calculated using Student's t-test. Values are shown as the mean and standard error of the mean (SEM). Each experiment was conducted three times and the data were pooled for analysis. Differences were considered to be statistically significant at *$p < 0.05$, **$p < 0.01$, or ***$p < 0.001$.

Ⅲ. RESULTS

1. TGs increases mRNA expression of TNF-α, but does not affect expression of IL-1β in Jurkat T lymphocytes

In order to investigate the effect of TGs on mRNA expression of TNF-α and IL-1β, pro-inflammatory cytokines that are deeply involved in the inflammatory response, TGs were treated to Jurkat T lymphocytes in a concentration-dependent and time-dependent manner. In Fig. 1, the amount of mRNA expression of TNF-α increased about two-fold over 24 h after exposure to TGs in Jurkat T cells. However, after 48 h, TNF-α is restored to a similar expression level before the treatment of TGs.

The expression level of IL-1β in Jurkat T lymphocytes showed a different trend in the response of TNF-α. In Fig. 2, the expression level of IL-1β did not change after 48 h of exposure to TGs in Jurkat T lymphocytes. These results indicate that TNF-α is increased within 24 h when Jurkat T lymphocytes are exposed to TGs. While IL-1β is not affected by TGs.

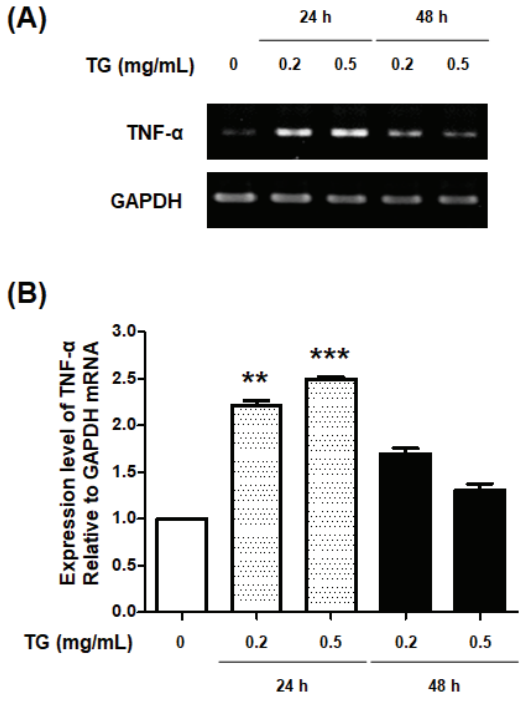

Fig. 1. RT-PCR for detection of TNF-α mRNA expression in the Jurkat T lymphocytes.

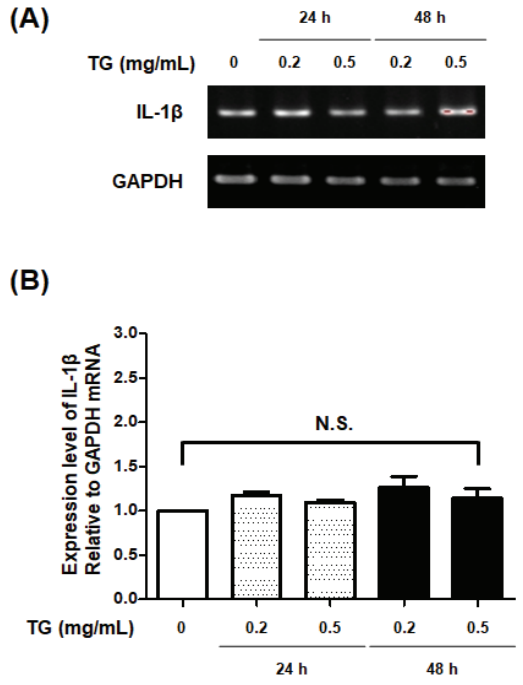

Fig. 2. RT-PCR for detection of IL-1β mRNA expression in the Jurkat T lymphocytes.

2. TGs increases both mRNA expression of TNF-α and IL-1β in U937 monocytes

Experiments were conducted to determine whether mRNA expression of TNF-α and IL-1β changes when U937 monocytes, one of the other immune cells that contribute to the inflammatory response, are exposed to TGs. In Fig. 3 and Fig. 4, unlike Jurkat T lymphocytes, the expression of TNF-α and IL-1β were increased when U937 cells were treated with TGs. However, in Fig. 3, the increase in expression of TNF-α in U937 monocytes was observed 48 h after exposure to TGs. In Fig. 4, the expression level of IL-1β increased more than 2-fold after 24 h of treatment with TGs and decreased to the level before exposure to TGs after 48 h. These results showed that the mRNA expression of IL-1β as well as TNF-α was increased when U937 monocyte was exposed to TGs. However, IL-1β was firstly increased after exposure to TGs, and TNF-α was increased after IL-1β was increased.

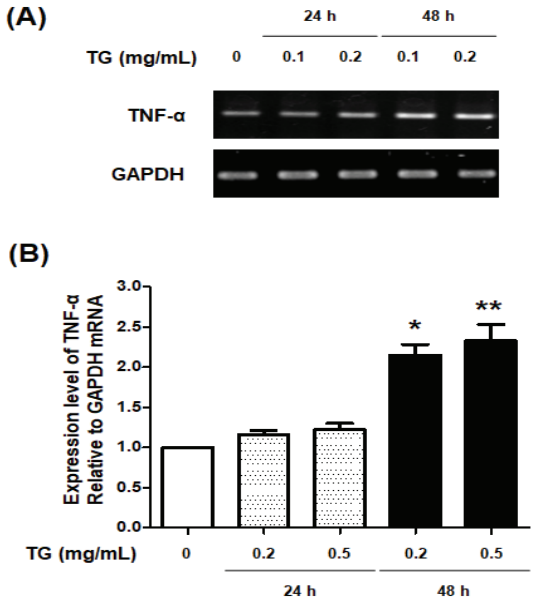

Fig. 3. RT-PCR for detection of TNF-α mRNA expression in the U937 cells.

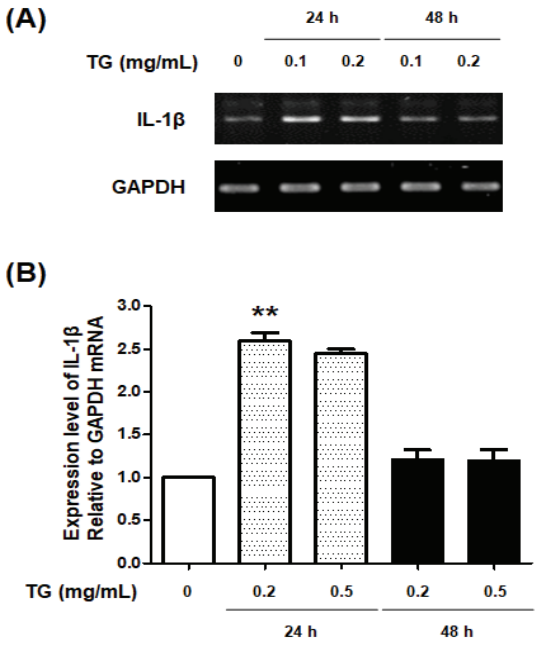

Fig. 4. RT-PCR for detection of IL-1β mRNA expression in the U937 cells.

3. The increase of pro-inflammatory cytokines by TGs occurs via the activity of iNOS in U937 monocytes and Jurkat T lymphocytes

Inhibitor studies were performed using iNOS inhibitor W1400 to determine whether iNOS is involved in the expression of TNF-α and IL-1β, which are pro-inflammatory cytokines that are increased by exposure to TGs.

In Fig. 5, in Jurkat T lymphocytes, mRNA expression level of TNF-α increased by exposure to TGs was decreased depending on iNOS inhibitor concentration.

In Fig. 6 and Fig. 7, in U937 monocytes, the increase in IL-1β and TNF-α mRNA expression by TGs was also inhibited by iNOS inhibitors. These results indicate that iNOS is involved in the mechanism of increased mRNA expression of TNF-α and IL-1β by TGs.

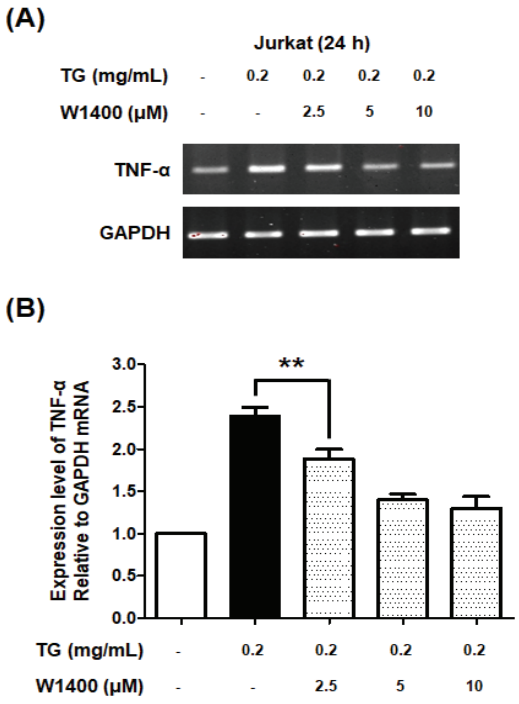

Fig. 5. RT-PCR for detection of TNF-α mRNA expression in the Jurkat T lymphocytes pretreated with iNOS inhibitors.

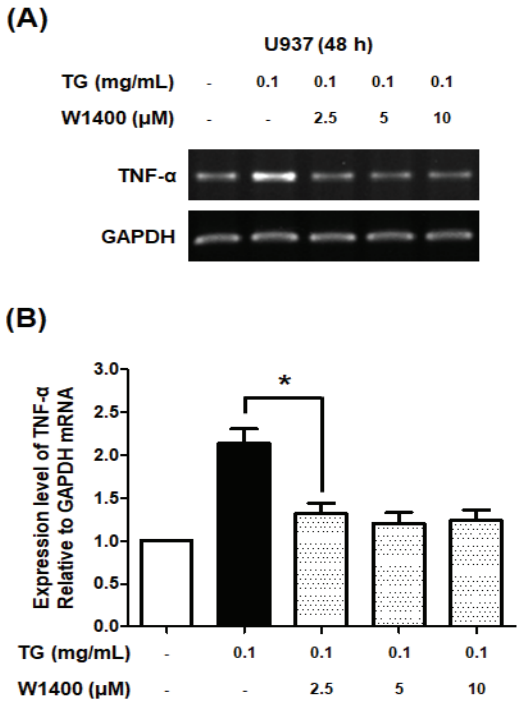

Fig. 6. RT-PCR for detection of TNF-α mRNA expression in the U937 cell pretreated with iNOS inhibitors.

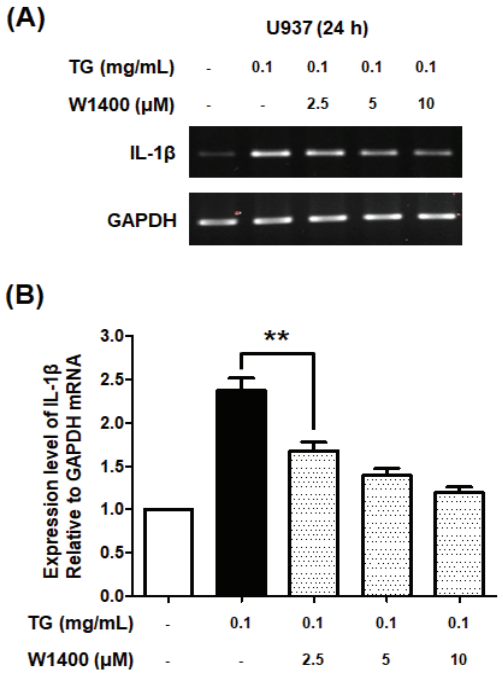

Fig. 7. RT-PCR for detection of IL-1β mRNA expression in the U937 cell pretreated with iNOS inhibitors.

Ⅳ. DISCUSSION & CONCLUSION

T lymphocytes and monocytes secrete a variety of cytokines in inflammatory diseases, but their pattern and mechanism of action differ depending on the disease. In this study, in Fig. 1, expression of TNF-α increased only 24 hours after TG treatment in Jurkat T cell (human T cell line). On the other hand, in Fig. 3 and Fig. 4, in U937 cells (human monocytic cell line), the expression of IL-1β was increased first 24 hours after TG treatment, and the expression of TNF-α was increased 48 hours after TG treatment. In other studies, caspase-1 activation via expression of IL-1β was markedly increased in monocytes. Unlike monocyte, T cell response was mainly mediated by TNF-α.[14,15]

However, in Fig. 5~7, TNF-α or IL-1β secretion by TG in T cells and monocytes was induced by iNOS. When TNF-α and IL-1β binds to receptors of various immune cells and stimulates the cells, the activity of nuclear factor kappa light chain enhancer of activated

B cells (NF-kB) is markedly increased, and then the expression of iNOS is increased.[16-18] iNOS is an enzyme that produces NO and is induced by cytokine, oxidative stress, and inflammatory response. NO produced by iNOS causes cell damage and induces inflammation and apoptosis.[19] Aronis et al.[16] reported that TG treatment increased reactive oxygen species (ROS) production in macrophages. The ROS produced in this way causes malfunction of mitochondria, exacerbating inflammation and ultimately inducing apoptosis.[20-22] Therefore, when iNOS is activated by TG, it may participate in the pathogenesis of atherosclerosis through this process.

TG induces inflammatory responses in the blood vessels, inducing various vascular diseases, and a typical disease is atherosclerosis. Thus, clarifying the role of TG helps to understand these diseases, but there are cases where it is necessary to consider the presence of TG in other diseases. For example, radiation therapy is a typical method of cancer treatment. Radiation therapy is a treatment for destroying cells by irradiating ionizing radiation such as X-rays, gamma rays, and electron beams to lesion sites. However, such ionizing radiation is known to be an inducer of iNOS activation when irradiated to T cells or mononuclear cells.[12,23] Therefore, irradiation with ionizing radiation to cancer patients with hyperlipidemia may cause a rapid increase in the inflammatory response due to excessive activation of iNOS. In such cases, side effects are likely to increase after radiation therapy. Therefore, when using ionizing radiation, the concentration of triglyceride in the blood of patients should be considered.

However, in this study, there is a limit to observing only the change in mRNA level in the cells. I wanted to examine in general whether the cytokine secretion by TG in Jurkat T cell and U937 monocyte is related to iNOS. To confirm this overall change, gene detection method is effective for determination of quickly changes within the cell after TG treatment. Although it is recommended to check the secretion and activity of proteins that actually work within the cell, there are many difficulties in determining the total mechanism in the cells. Also, since the expression of mRNA in genes is the last step of secreting proteins in cells, the mRNA change has a direct connection with changes in proteins. Therefore, it is believed that there is no problem in predicting the result even with mRNA detection. Of course, once the iNOS-related TG mechanism has been identified as a whole, it will proceed with further research to directly identify the proteins associated with genes.

Taken together, TG-induced iNOS activity act as a major control factor in inflammation and may contribute to further aggravating inflammatory lesions. On the other hand, if hyperlipemia and atherosclerosis are alleviated by inhibiting iNOS expression, iNOS may be used as an effective target material for patient treatment.

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Jurkat T 림프구와 U937 단핵구에서 중성지방 처리 시 iNOS를 통한 염증성 사이토카인의 mRNA 발현 증가

장정현

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요 약

중성지방(Triglyceride, TG)는 죽상동맥경화증과 같은 혈관의 만성 염증성 병변을 유발하는 인자 중 하나이다. 종양괴사인자-알파 (TNF-α), 인터루킨-1 베터 (IL-1β)와 같은 염증성 사이토카인은 염증 질환의 주요 요인으로 염증 부위에 T 림프구, 단핵구등의 면역 세포의 침윤을 유도하거나 세포 및 조직 괴사를 일으킴으로써 질병을 더욱 악화시킨다. 본 연구에서는 혈관 염증에 관여하는 Jurkat T 림프구와 U937 단핵구에 TG를 처리하였을 때 TNF-α와 IL-1β의 발현에 미치는 영향을 조사하고자 했다. Jurkat T 세포에서 TG에 의해 TNF-α의 mRNA 발현이 증가하였고, U937 단핵구에서는 TG에 의해 TNF-α와 IL-1β 모두 mRNA 발현이 증가하였다. 또한 유도성 산화질소합성효소(inducible nitric oxide synthase, iNOS)가 TG에 의한 TNF-α와 IL-1β의 발현 증가에 관여하는지 확인하기 위해 iNOS 억제제인 W1400을 세포에 전처리하여 iNOS의 활성을 차단하였다. 그 결과, W1400을 전처리한 세포에서는 TG에 의한 TNF-α 및 IL-1β mRNA 양이 대조군과 유사하게 낮은 수준으로 관찰되었다. 이는 혈관 내 TG의 증가가 T 림프구와 단핵구를 자극하여 iNOS 신호를 거쳐 염증성 사이토카인을 분비시키고 혈관염증질환을 발생하는데 관여하는 것을 확인시켜주었다. 결론적으로, 중성지방이 염증성 병변을 악화시키는데 있어 iNOS의 활성이 사이토카인 분비 등에 작용하며 병변을 더욱 악화시키는데 기여할 수 있다. 반면, iNOS 발현을 조절하여 고지혈증 환자의 치료에 유효한 표적물질로 이용될 가능성이 있다고 사료된다.

중심단어: 중성지방 (triglyceride, TG), TNF-α, IL-1β, iNOS

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