Antifibrotic Effects of Ethyl Pyruvate via Inhibition of HMGB1 on Keloid Fibroblasts and Keloid Spheroids

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(Directed by Professor Lee, Won Jai)

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October 2019
Youngdae LEE

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ABSTRACT

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Keloids are fibrous skin lesions; how keloids develop and treatment for them remain unclear. The etiology of keloids is characterized by an abnormally increased proliferation of cells, excessive accumulation of extracellular matrix, and reduction of apoptosis and autophagy. We investigated the role of high-mobility group box 1 (HMGB1) protein regulation in modulating the etiology of keloids. HMGB1 is a nuclear protein present in eukaryotic cells, known to regulate inflammation, immunity, and cell proliferation and death, and has been reported to be associated with various fibrous lesions. In particular, HMGB1 is known to regulate homeostasis between apoptosis and autophagy. In addition, effects of ethyl pyruvate, which is known to inhibit the extracellular action of HMGB1, on keloids with respect to the regulation of cell death by limiting the function of HMGB1 were examined.

Immunohistochemical staining confirmed that HMGB1 expression was increased in keloid tissues compared to that in normal tissues. Flow cytometry confirmed that autophagy was increased in fibroblasts treated with TGF-β and HMGB1. Furthermore, immunochemical staining verified that the expression of HMGB1 was significantly reduced by ethyl pyruvate treatment of keloid cells. Western blotting revealed that the expression of type 1, 3 collagen,

fibronectin, elastin, TGF-β, Smad 2/3, and ERK1 / 2 in keloid cells were significantly decreased by ethyl pyruvate treatment. Based on these results, autophagy was increased in keloids, whereas autophagy decreased, apoptosis increased, and fibrosis decreased with ethyl pyruvate treatment, an inhibitor of HMGB1. These results suggest that ethyl pyruvate may be applied for suppression and treatment of keloids.

Key words: High-Mobility Group Box 1 (HMGB1), Ethyl pyruvate, keloid, keloid spheroid

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I. INTRODUCTION

Keloids, considered to be benign fibrotic phagocytic tumors from abnormal skin fibrosis to their apex, are characterized by excessive deposition of extracellular matrix (ECM), mainly collagen fiber. As a rule, such tumors invade adjacent normal tissues and rarely spontaneously degenerate[1]. In recent years, interest in the field of pathological scars has increased, and various mediators have been found to affect the etiology of keloids; however, there is no clear understanding of the underlying mechanism of their development. The excess of ECM because of uncontrolled proliferation of keloid fibroblasts (KFs) is one of the best known causes involved in keloid development[2-7]. Thus, keloid formation has generally been assumed to be caused by an increased cell proliferation and decreased apoptosis rate in KFs[8-10]. Proper therapy may be related to inhibiting proliferation of KFs or reversing pathological fibrosis. Autophagy is a highly conserved cell death process that involves the breakdown of cellular components through lysosomal degradation. This contributes to maintaining cell homeostasis by disassembling and recycling unnecessary or damaged cell components[11]. In particular, autophagy is considered a pro-survival mechanism that adapts cellular subjects to stress, such

as malnutrition, prolonged inflammation, hypoxia, or chemotherapy. Thus, autophagy is associated with various human pathophysiologies, such as fibrosis, aging, tissue remodeling, and neurodegenerative diseases[12]. In particular, extensive research has been conducted on the importance of autophagy in cell homeostasis under stress; however, a single study that adequately deals with autophagy in pathological skin fibrosis, such as keloid formation, is lacking. As autophagy promotes cell viability even under stress conditions, we speculate that dysregulated cell death in keloids is associated with uncontrolled proliferation of KFs and development of keloids. The first major research question in this study is whether autophagy activity is altered in keloids. High-mobility group box 1 (HMGB1) is a ubiquitous nuclear protein that acts as a DNA chaperone that participates in DNA replication, recombination, transcription, and repair[13]. Upon cellular activation or injury, HMGB1 translocates to outside of the nucleus and is released into the cytosol or extracellular space. Overexpressed cytosolic HMGB1 is associated with increased cellular proliferation, mobility, angiogenesis, and resistance to apoptosis, whilst promoting autophagy and inflammation[14-19]. Thus, extracellular HMGB1 functions as a damage-associated molecular pattern, protein that activates the inflammatory response, and promotes cellular proliferation, differentiation, and migration[20]. All of these processes contribute to tumorigenesis, as well as pathologic fibrosis. Accordingly, recent evidence suggests that HMGB1 is involved in chronic inflammation, cancer, and various fibrotic diseases[13,14,21-27]. Thus, HMGB1 has been regarded as a key regulator of autophagy, because both cytosolic HMGB1 and extracellular HMGB1 enhance autophagic activity in response to cellular stress[19,28]. Because extranuclear HMGB1 promotes cell survival under stressed conditions by inducing autophagy, we sought to determine if HMGB1 is associated with keloid pathogenesis through regulation of the cellular death process[29]. Thus, we hypothesized that the inhibition of autophagic activity, while inducing apoptosis, would exert therapeutic effects on keloids. It has been shown that ethyl pyruvate, which is a derivative

of pyruvic acid, directly effects HMGB1 and inhibits its chemotactic and mitogenic activities in the extracellular space. Further, this compound has been shown to inhibit the cytoplasmic translocation of HMGB1[30,31]. The protective effects of ethyl pyruvate have been demonstrated on inflammation, pathologic fibrosis, and oncogenesis[29,31]. Consequently, inhibition of HMGB1 with ethyl pyruvate may disrupt keloid progression and attenuate fibrosis in keloids. In this study, we focused on the autophagic activity of keloids in regulating fibrogenesis, along with possible involvement of HMGB1. In addition, we highlighted the potential of ethyl pyruvate, a potent inhibitor of HMGB1, as a promising agent for the treatment of keloids.

II. MATERIAL AND METHODS

1. Preparation of cells

Normal human dermal fibroblasts (HDFs) and KFs were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA), penicillin (30 U/mL), and streptomycin (300 μg/mL). Cultures were maintained at 37°C in a humidified incubator under 5% CO_2, and the medium was changed every 2 days. In all experiments, cells were used before passage #7.

2. Preparation of keloid spheroids

Keloid and adjacent normal dermal tissues were obtained during a surgical procedure from patients with active-stage keloids. Keloid spheroids were prepared as described previously[32] by dissecting the central dermal tissue of the keloid into 2 mm-diameter pieces with sterile 21-gauge needles. Explants were plated onto HydroCell® 24 multi-well plates (Nunc, Rochester, NY, USA) and cultured for 4 hours in Iscove's modified Dulbecco's medium (Gibco)

supplemented with 5% fetal bovine serum, 10 μM insulin, and 1 μM hydrocortisone. Ethyl pyruvate, an inhibitor of HMGB1, was added into the plates containing keloid spheroids at 0, 1, 10, 20, and 40 mM, and incubated at 37°C in 5% CO_2 for 3 days. The treated keloid spheroids were then fixed with 4% formalin, paraffin-embedded, and cut into 5 μm-thick sections.

3. Methyl thiazolyl-diphenyl-tetrazolium bromide (MTT) assay

To assess cellular viability after ethyl pyruvate treatment of KFs and HDFs, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed, wherein 1×10^4 cells/cm^2 of KFs and HDFs were seeded in triplicate in 96 wells. After exposing the cells for 48 h to 0, 1mM, 5mM, 10nM, and 20mM of ethyl pyruvate (Sigma-Aldrich), 200 μL of a 0.5 mg/mL MTT solution (Boehringer, Mannheim, Germany) was added to each well and the plates were incubated at 37°C for 3 h. To dissolve the resulting formazan, 200 μL of dimethyl sulfoxide (Sigma-Aldrich) was added to each well after the MTT solution was removed. Absorbance was measured at 570 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

4. Histology and immunohistochemistry (IHC) of keloid spheroids

Keloid spheroids were treated with 0, 10, 20, and 40mM of ethyl pyruvate for 48 h. The spheroids were then washed, fixed with 4% formalin, paraffin-embedded, and cut into 5 μm-thick sections. Representative sections were stained with picrosirius red and then examined by light microscopy. For IHC staining, the keloid spheroid sections were incubated at 4°C overnight with mouse anti-HMGB1 (Abcam), mouse anti-collagen type I (Abcam), mouse anti-collagen type III (Sigma-Aldrich), mouse anti-elastin (Sigma-Aldrich), mouse anti-fibronectin (Santa Cruz Biotechnology), rabbit anti-transforming growth factor (TGF)-β (Abcam), collagenase inhibitor (MMP1) (Abcam), or rabbit anti-Smad 2/3 primary antibodies. Sections were then incubated at room temperature for 20 minutes with the Envision™ kit (Dako, Glostrup, Denmark) as a secondary antibody. Diaminobenzidine/hydrogen peroxidase (Dako)

was used as the chromogen substrate. All slides were counterstained with Meyer's hematoxylin. The expression levels of HMGB1, collagen type I, collagen type III, elastin, fibronectin, TGF-β, MMP1, and Smad 2/3 were semi-quantitatively analyzed using Metamorph® image analysis software. Results are expressed as the mean optical density of different digital images.

5. Western blotting analysis for collagen markers and profibrotic markers

Quantitative measurement of representative profibrotic makers, collagen I, collagen III, fibronectin, elastic, MMP1, and TGF-β was performed using western blotting. HDFs, KFs, and KFs treated with 0mM, 10mM, 20mM, and 40mM ethyl pyruvate (cells were seeded at 10^5 cells/well) were cultured in 100 mm × 20 mm dishes for 48 h. Cells were lysed in 50 mM Tris-HCl (pH 7.6), 1% Nonidet P-40, 150 mM NaCl, and 0.1 mM zinc acetate in the presence of protease inhibitors. Protein concentrations were determined by the Lowry method (Bio-Rad), and 20 μg of each sample was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were then electrophoretically transferred onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA). The membrane was blocked with blocking buffer for 1 h and incubated overnight at 4°C with primary antibodies against collagen I, collagen III, fibronectin, elastic, MMP1, and TGF-β. After 2 h of incubation at room temperature with the secondary antibody (horseradish peroxidase-conjugated anti-rabbit, or anti-mouse; Santa Cruz Biotechnology), protein bands were visualized using chemiluminescence reagents (Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's instructions. Protein expression was analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA).

III. RESULTS

1. Ethyl pyruvate reduces human dermal fibroblast proliferation

To determine whether inhibition of HMGB1 with ethyl pyruvate affected human dermal cell proliferation, we performed an MTT assay. To assess cellular viability after ethyl pyruvate treatment, various concentrations of ethyl pyruvate (0, 1 mM, 5 mM, and 10 mM) were applied to HDFs 48 h before the MTT assay. The results showed a significant decrease in HDF proliferation after treatment with all tested concentrations of ethyl pyruvate (Figure 1). These results suggest that ethyl pyruvate reduces the viability of HDF cells.

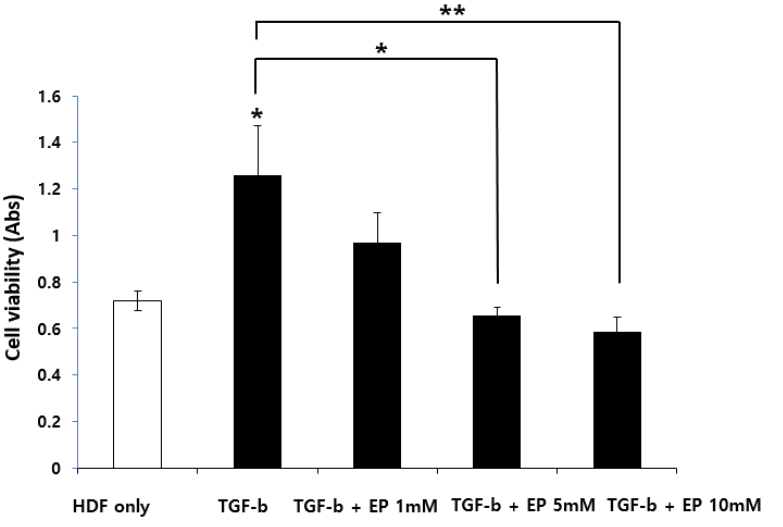


Figure 1. Effects of ethyl pyruvate on viability of normal human dermal fibroblasts (HDFs): MTT cell proliferation assay showed that ethyl pyruvate significantly inhibited the proliferation of HDFs.

2. Ethyl pyruvate reduces keloid fibroblast proliferation

To determine whether inhibition of HMGB1 with ethyl pyruvate affects keloid fibroblast cell proliferation, we performed an MTT assay. To assess cellular viability after ethyl pyruvate treatment, various concentrations of ethyl pyruvate (0, 10 mM, and 20 mM) were applied to KFs 48 h before the MTT assay. The results showed a significant decrease in KF proliferation after treatment with all tested concentrations of ethyl pyruvate (Figure 2). These results suggest that ethyl pyruvate reduces the viability of KF cells.

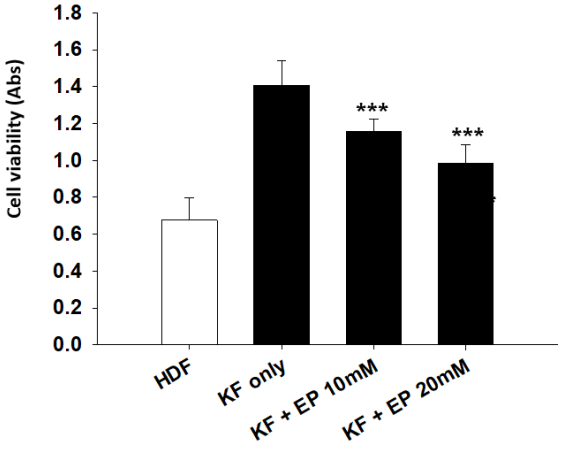


Figure 2. Effects of ethyl pyruvate on viability of keloid fibroblasts (KFs): MTT cell proliferation assay showed that ethyl pyruvate significantly inhibited the proliferation of KFs.

3. Ethyl pyruvate reduces HMGB1 expression in HDFs

If exogenous HMGB1 promotes autophagic activity in keloids, then inhibition of HMGB1 should reduce cellular viability of keloids. We assessed this hypothesis by treatment with ethyl pyruvate, which suppresses HMGB1 extracellular activities, as well as inhibits cytoplasmic translocation of HMGB1. Although ethyl pyruvate is recognized as a potent HMGB1 inhibitor, no study has investigated the effect of this compound on keloids. Therefore, we used keloid spheroids to assess whether ethyl pyruvate could reduce HMGB1 expression in keloids. We generated keloid spheroids following an established protocol[32] to mimic the keloid microenvironment. After treatment of keloid spheroids with various concentrations of ethyl pyruvate (0, 10, 20, and 40 mM), IHC staining for HMGB1 was performed. As shown in Figure 3, non-treated keloid spheroids showed a higher expression of HMGB1, whereas ethyl pyruvate-treated keloid spheroids showed a markedly decreased HMGB1 expression. The data were graphed using Metamorph® image analysis software. The results showed significantly decreased HMGB1 expression in keloid spheroids treated with 10, 20, or 40 mM of ethyl pyruvate.

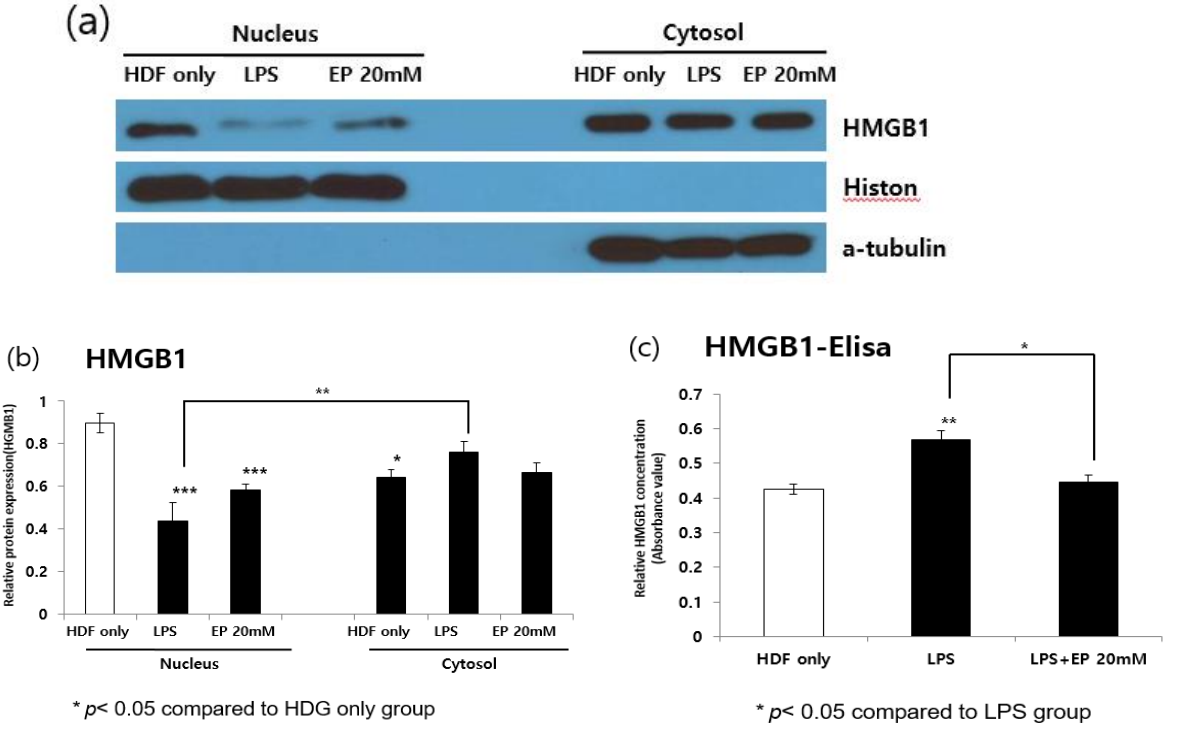
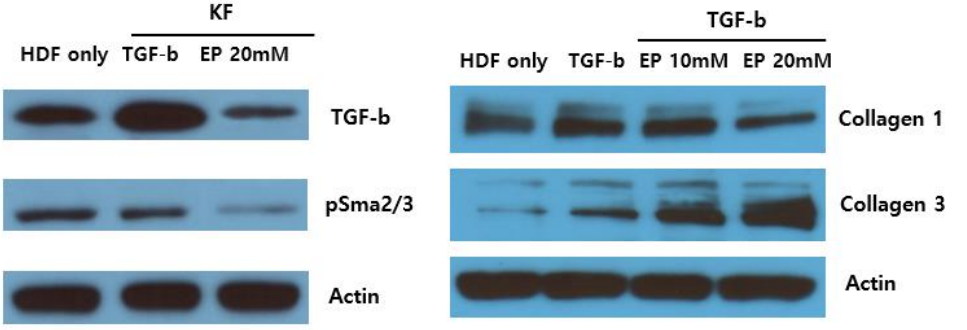


Figure 3. Effects of ethyl pyruvate on HMGB1 expression in keloid spheroids (a) Western blot was used to identify HMGB1 in keloid spheroids. Following the addition of ethyl pyruvate, the density of the HMGB1 was notably decreased in keloid spheroids (b). Semi-quantitative analysis indicated significantly decreased HMGB1 in ethyl pyruvate (20mM)-treated keloid spheroids versus non-treated keloid spheroids (c).

4. Ethyl pyruvate effects collagen I and III, TGF-β, and p-smad 2/3 in KFs and keloid tissue

To assess the consequence of ethyl pyruvate-induced autophagic cell death in keloids, we examined changes in collagen I and III, TGF-β, and p-smad 2/3 expression in fibroblast cell types by western blot. The western blot results indicated that the levels of collagen I and III, TGF-β, and p-smad 2/3 conversion rate in KFs were markedly higher, by 1.2 or 1.1 fold, in comparison with that in HDFs. Further, the significantly enhanced levels of collagen 3 and TGF-β in KFs compared with that in HDFs were notably increased following treatment with 20mM ethyl pyruvate. These results are concordant with those of the IHC data for keloid tissue that revealed a significant decrease in collagen 3 and TGF-β after ethyl pyruvate treatment (Figure 4-1). Collagen I and p-smad 2/3 expression levels in keloid tissues treated with ethyl pyruvate (20mM) were significantly reduced by 18.11% and 24.64%, respectively, in comparison with that in non-treated keloid tissue (***$p < 0.001$, Figure 4).



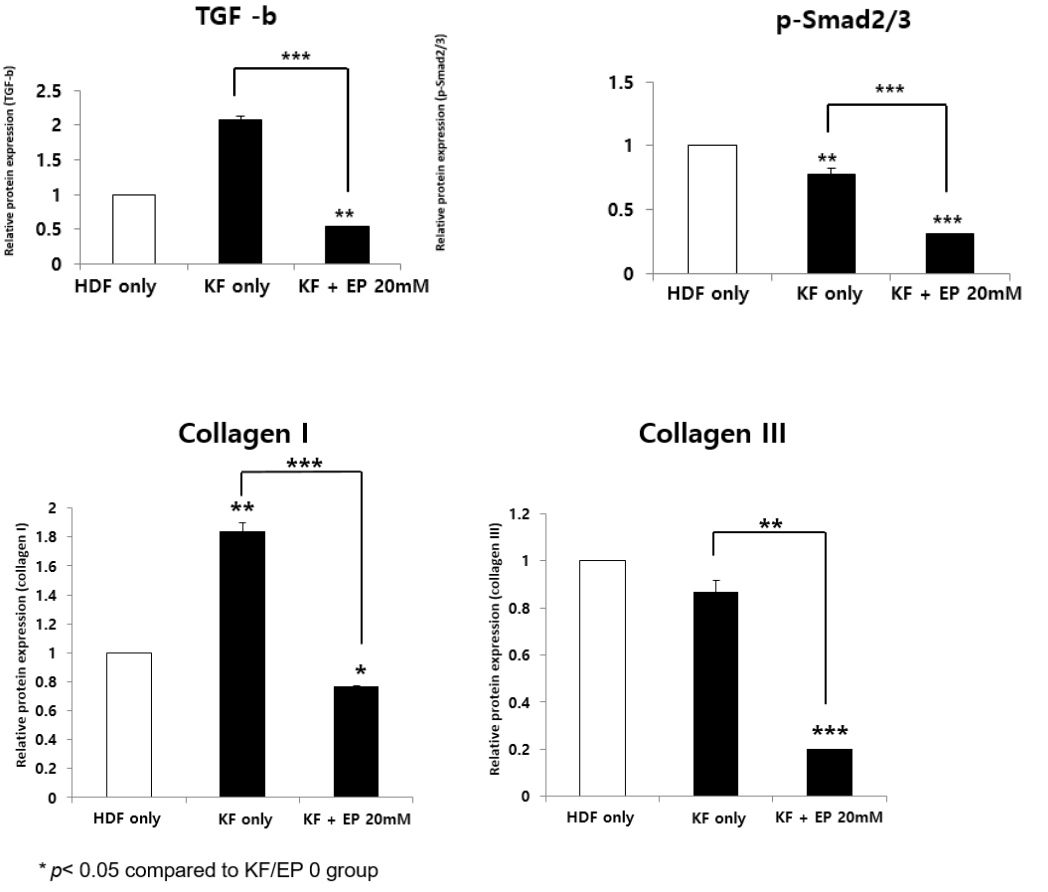
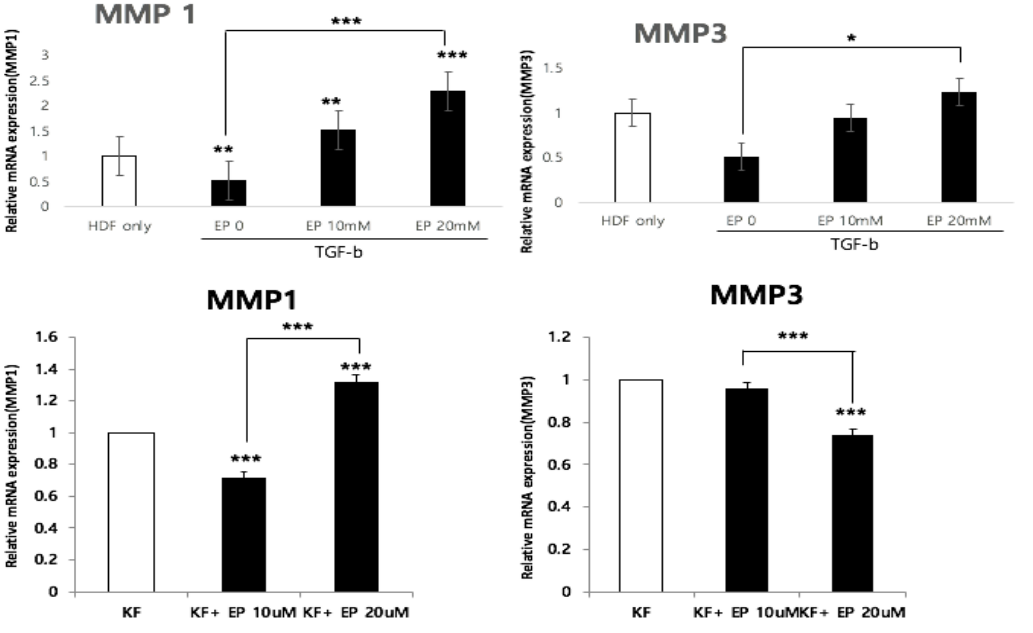


Figure 4. Effects of ethyl pyruvate on the expression of fibrotic markers in human dermal fibroblasts. Collagen I and p-smad 2/3 decreased after ethyl pyruvate treatment. Western blot analysis of fibrotic markers in HDFs showed that TGF-β and collagen III levels are increased in HDFs. HDFs show significantly decreased fibrotic markers after treatment with 20mM of ethyl pyruvate. (*$p < 0.05$, **$p < 0.01$).

5. Ethyl pyruvate treatments affect MMP-1 and -3 mRNA expression in HDFs and KFs, and analysis of the MMP-1 mRNA/TIMP1 mRNA ratio

We investigated the effects of ethyl pyruvate on the expression of metalloproteinase I (MMP1) and metalloproteinase III (MMP3) in HDFs and KFs. Matrix metalloproteinases (MMPs) have been directly implicated in keloids because they degrade extracellular proteins. In particular, the expression of tissue inhibitors of metalloproteinase I (TIMP-1) is upregulated leading to the inhibition of MMP activity and subsequent accumulation of matrix proteins in the extracellular space. MMP1 and MMP3 mRNA levels increase sequentially according to the concentration of ethyl pyruvate in HDFs. Additionally, the MMP1 mRNA levels increased in KFs, and MMP3 mRNA levels did not show the reverse effect. The MMP1/TIMP1 ratio did not show a constant trend in HDFs and did not show significant results. However, in KFs, there was a tendency to increase as the concentration of ethyl pyruvate increased, but the results were not significant.



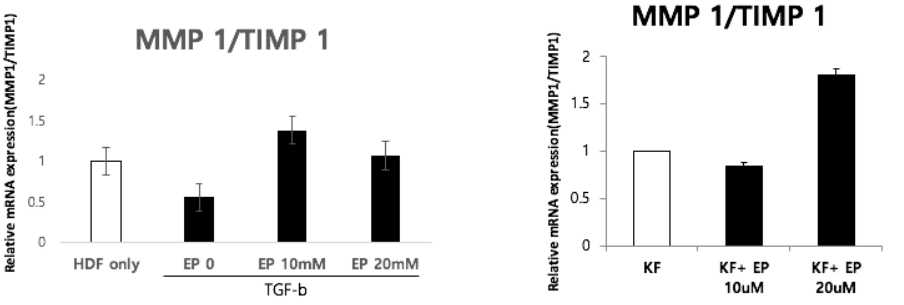
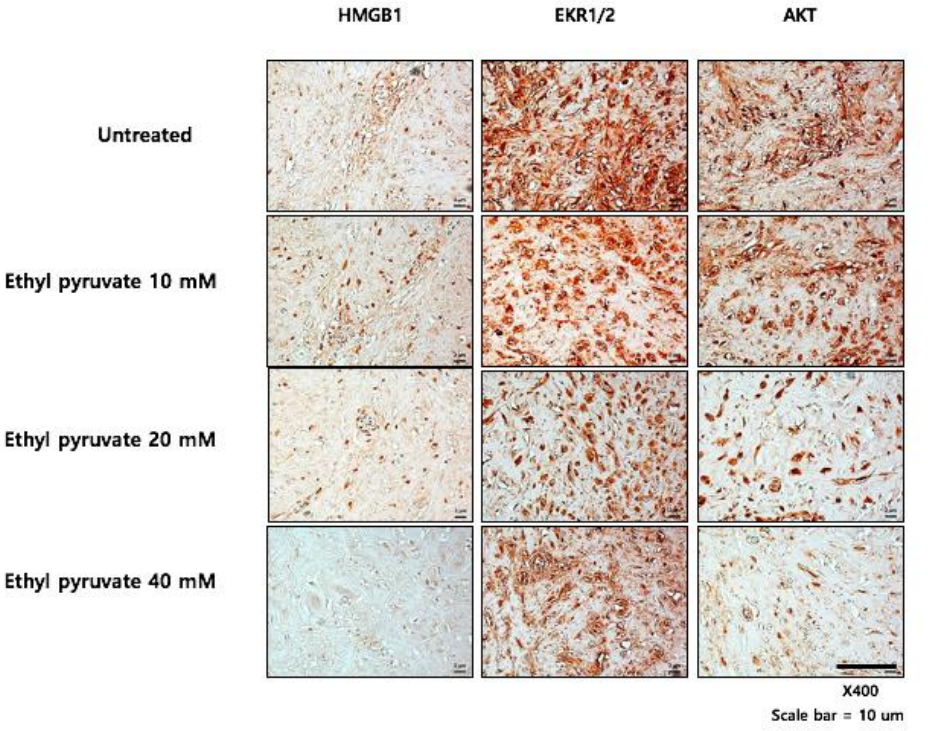


Figure 5. Effects of ethyl pyruvate on the expression of MMP1 and MMP3 in human dermal fibroblasts and keloid fibroblasts. MMP1 and MMP3 mRNA levels in HDFs increased sequentially according to the EP level (0mM, 10mM, and 20mM). MMP1 mRNA levels in KFs increased with the EP level. Other parameters and the ratio were not related with the EP concentrate level. (*$p <$ 0.05, ***$p <$ 0.01).

6. Ethyl pyruvate suppresses HMGB1, EKR1/2, and AKT expression in keloid spheroids

We investigated the effects of ethyl pyruvate on HMGB1, EKR1/2, and AKT expression. These molecules are quantitative profibrotic markers. Profibrogenic signaling molecules involved in collagen synthesis and cellular proliferation, such as ERK1/2 and AKT, were assessed by western blot analysis. We found significant changes in the expression levels of these molecules in HMGB1-treated HDFs. As shown in Figure 6, markedly decreased expression of all of the factors was observed after simultaneous treatment with ethyl pyruvate (10mM, 20mM, 40mM) (*p < 0.05; Figure 6). These results indicated that ethyl pyruvate reversed the action of HMGB1 and inhibited profibrotic signaling.



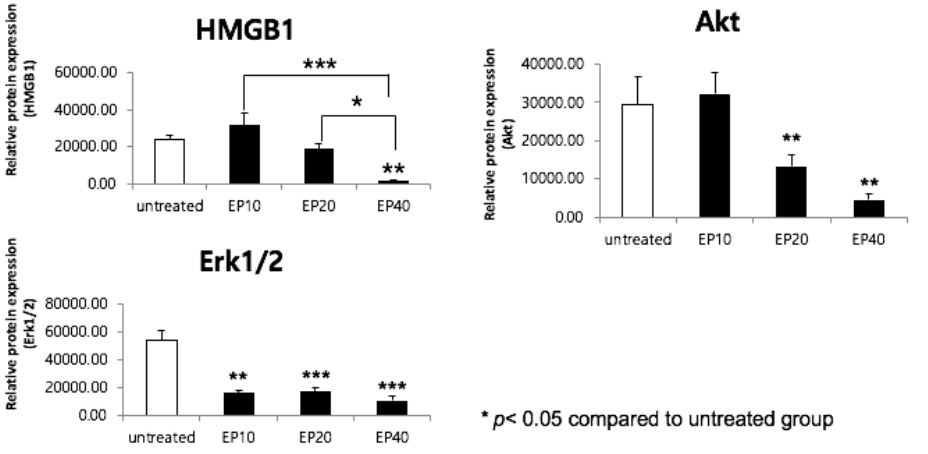
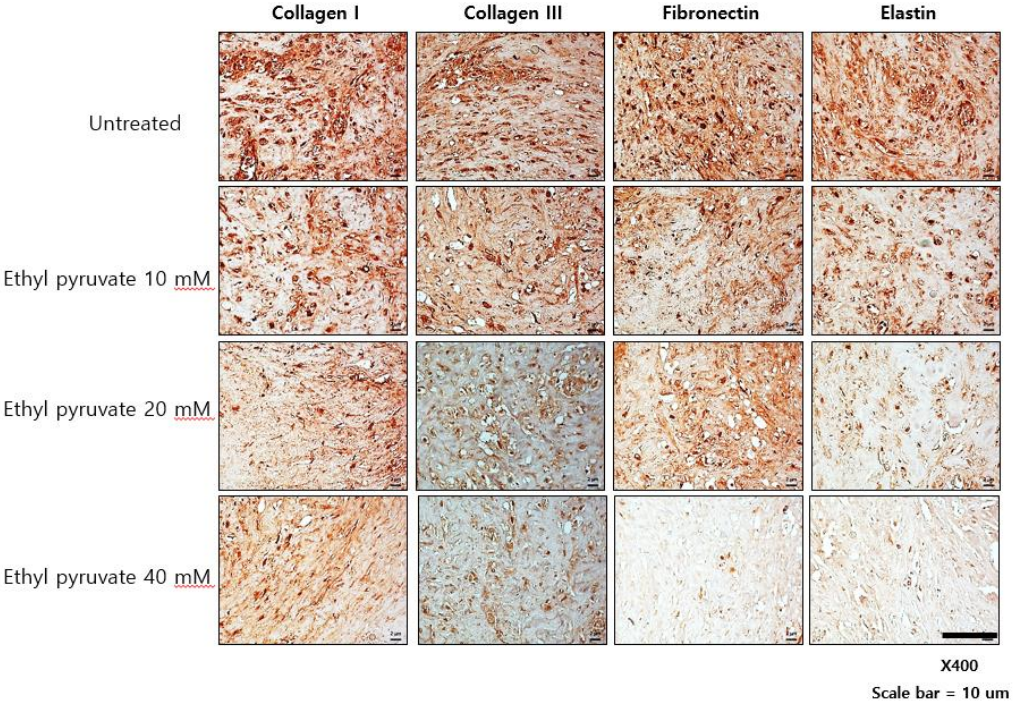
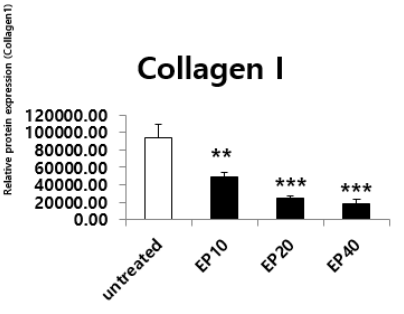


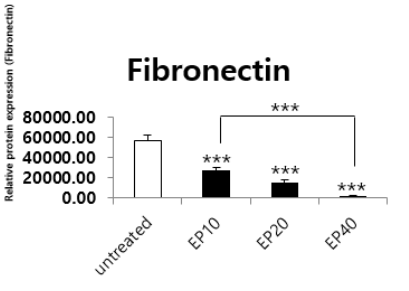
Figure 6. Effects of ethyl pyruvate on the expression of profibrotic factors in human dermal fibroblasts. HMGB1, ERK1/2, and AKT expression was significantly decreased after ethyl pyruvate (10mM, 20mM, and 40mM) treatment simultaneously with HMGB1 (100 ng) (*$p < 0.05$)

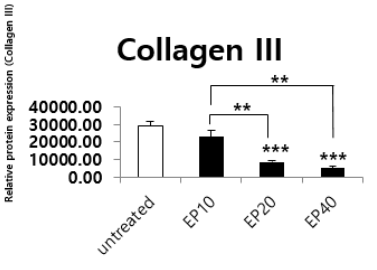
7. Ethyl pyruvate suppresses collagen I and III, fibronectin, and elastin in keloid spheroids

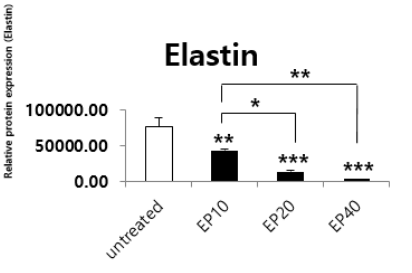
We further investigated the effects of ethyl pyruvate on collagen I and III, fibronectin, and elastin expression; these molecules are crucial regulators of fibrogenesis. Immunohistochemical staining revealed significantly reduced collagen I and III, fibronectin, and elastin levels in ethyl pyruvate (40mM)-treated keloid spheroids by 05.67%, 64.81%, 63.42%, and 62.30%, respectively, versus that of non-treated keloid spheroids ($p < 0.001$, Figure 5). Collectively, these data suggest that ethyl pyruvate modulates TGF-β and its signaling pathway, thereby reducing fibrosis in keloids.











* p< 0.05 compared to untreated group

Figure 7. Histochemical analysis of collagen I and III, fibronectin, and elastin in ethyl pyruvate-treated keloid spheroids. Representative images of collagen I and III, fibronectin, and elastin IHC staining of keloid spheroids treated with ethyl pyruvate (10, 20, and 40mM). Collagen I and III, fibronectin, and elastin were significantly decreased in keloid spheroids following ethyl pyruvate application.

IV. DISCUSSION

Keloids, which represent human fibrotic disorders characterized by dermal fibrotic proliferative tumors, extend beyond the boundaries of the original scar and invade adjacent normal skin[33]. Although various factors influence the development of keloids, excessive ECM accumulation because of the over-proliferation of KFs and the difficulty in regulating apoptosis are the major pathophysiological factors involved[34]. Autophagy is a form of cell death that involves lysosomal degradation and recycling of damaged or excessive organelles. Autophagy is a process of cell death, but there is increasing evidence that this process acts as a protective cellular mechanism that ensures proper energy metabolism under stress conditions, such as starvation, oxidative stress, hypoxia, and anticancer therapy[35-39]. Thus, we hypothesized that autophagy activity was associated with the development of keloids[40]. In various microenvironments, the increase and decrease of autophagy play an important role in the pathogenesis of diseased tissue[36]. Keloids and hypertrophic scar tissue look clinically similar, but their molecular basis and clinical behavior are quite different. For example, they exhibit distinct sensitivity to different apoptotic cell death pathways and KF growth factors. HMGB1, a ubiquitous and abundant nuclear protein, has chemotactic and mitotic activity in inflammatory cells and fibroblasts[41]. New evidence suggests that HMGB1 is involved in pathological fibrosis, which affects various organs of the human body, including tumor formation, as well as the regulation of inflammation, tissue fibrosis, immune responses, and cell death[41-43]. Cytoplasmic translocation of HMGB1 promotes autophagy and limits programmed atherosclerosis cell death.

Endogenous HMGB1 regulates the balance of apoptotic self-predation. Cellular stress promotes HMGB1 release from cells and the HMGB1 promotes autophagy flux[41]. Therefore, we speculate that HMGB1 is associated with abnormal cell death in keloids exhibiting weakened apoptosis activity. Thus, we confirmed the presence and overexpression of HMGB1 in human keloid tissue. In addition, enhanced autophagy activity was confirmed in HDF treated with exogenous HMGB1 or TGF-β, leading to a fibrous state. Subsequently, we inhibited HMGB1 activity and observed changes in factors related to fiber death and fibrosis in keloids. Recent evidence has shown that ethyl pyruvate, which binds directly to HMGB1, impairs extracellular activity, inhibits extracellular release, and reduces the chemical attractant and mitotic activity of HMGB1. Here, we showed that ethyl pyruvate inhibited mitotic activity in HMGB1-expression-attenuated keloids. In agreement with previous studies using ethyl pyruvate as an HMGB1 inhibitor in fibrotic disease, we showed that this compound improves fibrosis of keloid spheroids. TGF-β is a crucial factor in the proliferation and collagen synthesis in keloids because it enhances the mitogenic response[44-46]. Pivotal mediators of the TGF-β signaling pathway, and Smad 2/3 and ERK1/2 complexes, are highly activated in keloids and have been implicated in keloid pathogenesis[47]. We found that the expression of TGF-β and the Smad 2/3 and ERK1/2 complexes were significantly attenuated by ethyl pyruvate in keloid spheroids. Together, these results revealed that ethyl pyruvate exerts a potent antifibrotic effect on keloids. The direct inhibitory effect of ethyl pyruvate on HMGB1 is already well known, ethyl pyruvate possesses various pharmacological and biological activities against inflammation, oxidative stress, and tumorigenesis, suggesting that the present effects may not be solely attributable to the inhibitory effect of HMGB1[48]. Further, the inhibition of autophagic activity in keloids was not the only contributing factor to the antifibrotic action of ethyl pyruvate. The HMGB1 blocker, ethyl pyruvate, was shown to ameliorate fibrosis in keloids. This effect may result from the inhibition of the TGF-β-related pathways, as well as regulation of the cell death process.

V. CONCLUSION

Inhibition of HMGB1 by ethyl pyruvate reduces fibrosis and autophagy and increases apoptosis in keloids. These results represent a new strategy for keloid treatment targeting HMGB1-mediated fibrosis.

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ABSTRACT (IN KOREAN)

켈로이드 섬유 아세포 및 스페로이드에서 HMGB1 억제를 통한 Ethyl pyruvate의 항 섬유 효과

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이영대

켈로이드는 발생기전 및 치료방법이 명확하게 밝혀지지 않은 섬유성 피부 병변이다. 켈로이드의 병인은 비정상적으로 세포의 증식이 증가되어 세포 외 기질이 과도하게 축적되며 자멸사 및 자가 포식 역시 감소하는 것을 특징으로 한다. 우리는 이와 같은 켈로이드의 병인을 조절하기 위해 High mobility group box 1 (HMGB1) 단백질 조절을 선택하였다. HMGB1은 진핵 세포에 존재하는 핵 단백질로 염증, 면역, 세포의 증식 및 사멸 등을 조절하는 것으로 알려져 있으며, 여러가지 섬유성 병변과 관련되어 있는 것으로 보고되었다. 특히 HMGB1은 세포 자멸과 자가 포식과의 항상성을 조절하는 것으로 알려져 있으며 우리는 켈로이드의 병인이 자멸사와 자가 포식의 감소와 연관되어 있는지 선행연구를 통해 확인하였다. 또한, HMGB1의 세포 외 작용을 억제하는 것으로 알려진 Ethyl pyruvate을 이용하여 keloid에서 Ethyl pyruvate가 HMGB1의 역할을 제한하여 세포 사멸의 균형에 미치는 영향을 알아보고 이를 통한 항섬유화 효과를 확인해 보고자 하였다.

면역화학염색을 통해 정상조직에 비해 keloid 조직에서 HMGB1의 발현이 증가함을 확인하였고, 선행연구를 통해 포분석을 통해 대표적 섬유성 인자인 TGF-β와 HMGB1을 처리한 섬유 모세포에서 자가 포식이 증가함을 확인하였다. 켈로이드 세포에 Ethyl pyruvate를 처리하여 HMGB1의 발현이 감소함을 면역화학염색을 통해 확인하였으며, MTT 분석을 통해 정상 섬유 모세포와 켈로이드 섬유 모세포에서 Ethyl pyruvate (0, 10, 20mM) 처리 후 세포의 증식이

유의하게 감소함을 확인하였다. 켈로이드 세포구에서 type 1, 3 collagen, fibronectin, elastin과 TGF-β, Smad2/3, ERK1/2의 발현이 Ethyl pyruvate 처리에 따라 유의하게 감소함을 western blot을 통해 확인하였다. 이 결과들을 토대로, 켈로이드에서 자가 포식이 증가되어 있고, HMGB1의 억제제인 Ethyl pyruvate의 처리에 따라 자가 포식이 억제되고, 세포 자멸이 증가하며, 섬유화가 감소하는 결과를 확인하였다. 이를 통해 keloid의 억제 및 치료방법 개발에 응용할 수 있을 것으로 사료된다.

핵심되는 말: High Mobility Group Box 1 (HMGB1), Ethyl pyruvate, 켈로이드, 켈로이드 스페로이드