Is leukotriene B4 one of the keloid marker? a fibroblast keloid study

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Abstract

Keloid pathogenesis occurs due to the duration of prolonged inflammatory phase and increased production of various growth factors such as TGF-β1 which may cause increasing fibroblast proliferation and collagen synthesis. Existence of one of the chemical mediators released during inflammation, leukotriene B4 (LTB4), in keloid pathogenesis specifically in the phases of inflammation and proliferation, is still unclear. The purpose of the study is to analyze the levels of LTB4 in keloid. Methods: Fibroblast culture that was done by explanting keloid and normal skin of a keloid patient. Measurement of LTB4 on keloid and normal fibroblast was done by Elisa method. This experiment was run in triplicate. Statistical test was conducted by t test for the unpaired data and Anova test. The experiment was done at cell culture laboratory of Medical Faculty of Padjadjaran University Bandung. Levels of LTB4 in keloid fibroblast was higher than that of normal skin fibroblast (mean 23143.27 vs 18191.85 pg/ml; p<.05). Conclusion, increased LTB4 levels in keloid fibroblast showed the existence of LTB4 role in the prolonged inflammatory process in keloid pathogenesis.

Introduction

A keloid is a scar tissue that is developed due to the abnormal response in the wound healing process. Moreover, a keloid is formed as a result of increasing fibroblast production and collagen synthesis, hence it being one of fibroproliferative disorders.1-3 Keloid incidents, in which estimated 15% to 20%, accounts for having been suffered by Afro-Americans, Hispanics, and Asians.4,5 Keloid pathogenesis occurs as a result of longer inflammation and the increasing production of various growth factors such as Platelet Derived Growth Factor (PDGF), Transforming Growth Factor-β (TGF-β), and Vascular Endothelial Growth Factor (VEGF). Transforming Growth Factor-β (TGF-β) acts in fibroblast proliferation. One of the responses to the abnormal wound healing is the longer duration of inflammation phase.1,6 Due to the cell membrane damage, phospholipids as the cell membrane structure would release arachidonic acid, supported by phospholipase enzyme. During the inflammation phase, mediators of inflammation would be released, including the lipid mediator that is leukotriene (LTs).1,4,7 Leukotriene B4 (LTB4) is a potent lipid mediator of inflammation. The main functions of LTB4 is to be leukocyte chemoattractant and to act in the inflammation pathogenesis, immune disorders, and fibrosis.7,8 An in vitro study shows that LTB4 is a chemoattractant for fibroblast and a regulator of fibroblast influx into the tissue.9,10 Leukotriene B4 could also be found on skin disorders such as atopic dermatitis, psoriasis, and systemic sclerosis. The existence of one of the chemical mediators released during inflammation, LTB4, in keloid pathogenesis specifically in the phases of inflammation and proliferation, is still unclear.7,8

Materials and Methods

The study was based on the approval of the Ethic Commission of the Faculty of Medicine of Padjadjaran University in Bandung. There was only one patient whose keloid tissue to be taken. Keloid excision was done on the patient according to the standard operating procedure (SOP). The decision of the keloid diagnosis was taken by a dermatologist. As the tissue excision was completed, the next procedure being the preparation for the primary cell culture with skin explant method. The study utilized the popular medium for the fibroblast transport and growth, Dulbecco’s Modified Eagle Medium (DMEM), shot with penicillin, streptomycin, and fetal bovine serum (FBS) or fetal calf serum (FCS). The study took place in the Cell Culture Research Laboratory of the Faculty of Medicine of Padjadjaran University/Hasan Sadikin Hospital in Bandung. The study materials consisted of the keloid fibroblast from the primary culture of keloid tissue and fibroblast culture of a patient’s normal skin. The fibroblast culture applied in the study was the third subculture while the study objects included LTB4 ELISA kit. The inclusion criteria of the study consist of patient’s keloid which had never received any treatments and > 80% of cell viability on primary culture while the exclusion criteria was the cells grown on the culture, not on fibroblasts. Fibroblast culture that was done by explanting keloid and normal skin of a keloid patient. The measurement of LTB4 on keloid and normal fibroblast was done by Elisa method. This experiment was run in triplicate. The statistical test was conducted by t test for the unpaired data and Anova test. The experiment was done at the cell culture laboratory of Medical Faculty of Padjadjaran University Bandung. The study utilized in vitro experimental study design with the comparative completely randomized design.

Results

The result of fibroblast cell culture showed keloid fibroblast grew more rapidly than normal skin fibroblast. Figure 1 showed keloid fibroblast started to grow from explant periphery while in normal skin, fibroblast grew on day 10. Figure 2 showed the growth of fibroblasts in keloid and Figure 3 showed normal skin reached 80% confluence, hence the subculture (Figure 4). Such result prompted the second stage of study. In this study, the LTB4 level in keloid fibroblast was higher than normal skin fibroblast, as seen on Table 1.
Discussion

Cell culture has been commonly used and is a method required for studies of wound healing process. Fibroblast cell culture with skin explant method is a primary culture, produces fewer fibroblasts despite being more commonly used due to its better cell morphology and fewer risks on cell damage as a consequence from enzymatic process. Fibroblast has an important role in the wound healing process. One of the abnormal wound healing processes could induce tissue fibrosis similar to that in keloid. Keloid occurs due to the abnormal wound healing process comprising elongation in proliferative phase and delayed remodeling. In addition, a theory has stated that keloid occurs due to the prolonged inflammatory phase. Leukotriene is one of the arachidonic acid metabolites, which is released through 5-lipoxygenase pathway (5-LO). Leukocytes are the main source of leukotriene. However, other cells such as macrophages, monocytes, fibroblasts, epithelial cells, and platelets are also able to produce leukotrienes. Leukotriene B4 is a potent lipid mediator released by the activation of neutrophils, macrophages, and mast cells; as well as a chemotactic factor that is a fibroblast chemotactic factor. In prolonged inflammation, such cells would stay throughout the wound healing process with the total amount of macrophages and leukocytes higher in keloid fibroblast than those in normal fibroblast. The level of leukotriene B4 increases in several skin disorders such as atopic dermatitis, psoriasis, and systemic sclerosis though based on the researcher’s knowledge, there has been no prior studies on the LT4 level in keloid. In the study, the level of LT4 in keloid fibroblast was significantly higher than normal skin fibroblast with the significant value (2-tailed) of LT4 0.01 less than the p value <0.05 as seen on Table 1. The study result is in tune with a theory stating that keloid pathogenesis occurs as a consequence of prolonged duration of inflammation phase. In a chronic inflammation, cells involved in the mechanism of inflammation would remain, including neutrophil, therefore the increase of LT4 level could also be found in chronic inflammation. Further research is required to explore the role of LT4 in keloid pathogenesis. The limitations in this study consisted of the sample which was being taken from the patient’s keloid by elliptical excision; there has been no clear macroscopic boundary to differ normal skin from keloid, therefore during the excision, there was an open possibility for a part of keloid to have been carried into the normal tissue.

Conclusions

Increased LT4 levels in keloid fibroblast showed the existence of LT4 role in the prolonged inflammatory process in keloid pathogenesis. Further research with more sample is required to explore the role of LT4 in keloid pathogenesis.

Table 1. The Comparison of LT4 Levels in Keloid and Normal Skin Fibroblasts.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal</th>
<th>Keloid</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT4 (pg/mL)</td>
<td>18191.85</td>
<td>23143.27</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean</td>
<td>18191.85</td>
<td>23143.27</td>
<td></td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>1176.83</td>
<td>1782.15</td>
<td></td>
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<tr>
<td>Std. Error mean</td>
<td>679.44</td>
<td>1028.92</td>
<td></td>
</tr>
</tbody>
</table>

Note: *based on unpaired t-test (p<0.05).

References

7. Sharma JN, Mohammed LA. The Role of leukotrienes in the pathophysiology of inflammatory disorders: Is there a case for revisiting leukotriene as therapeutic targets?. Inflammopharma-