Determination of TGFβ1 activity in placental extracts

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Abstrak

Penelitian ini dilakukan untuk menyelidiki aktivitas dan pengaruh prosedur ekstraksi terhadap aktivitas TGFβ1 dalam extrak plasenta, setelah direbus selama 30 menit, tidak dipanaskan dan dipanaskan pada 70°C selama 30 menit. Proses asidifikasi juga dilakukan untuk mendapatkan aktivitas total. Hasil penelitian menunjukkan bahwa : (1) Aktivitas TGFβ1 bebas sebelum direbus berkisar antara 10±7 - 22±9 pg/mg protein, sedangkan aktivitas total TGFβ1 adalah 100±81 - 240±51 pg/mg protein (tahap I dan II extraksi). (2) Aktivitas TGFβ1 bebas setelah direbus (tahap III) tidak terdeteksi, tetapi setelah asidifikasi untuk menghilangkan latensi ditemukan aktivitas TGFβ1 total dalam ekstrak plasenta terakhir setinggi 165±120 pg/mg protein. (3) Pada plasenta yang tidak dipanaskan, didapatkan aktivitas TGF1 bebas setinggi 95 ± 52 pg/mg protein, sedangkan aktivitas TGFβ1 total adalah 838 ± 240 pg/mg protein (tahap III). Aktivitas TGF1 bebas dalam ekstrak plasenta terakhir adalah setinggi 6 ± 2 pg/mg protein, sedangkan aktivitas TGFβ1 total adalah 55 ± 13 pg/mg protein. (4) Pada plasenta yang dipanaskan pada 70°C, didapatkan aktivitas TGFβ1 bebas setinggi 12 ± 4 pg/mg protein, sedangkan aktivitas TGFβ1 total adalah 379 ± 238 pg/mg protein (tahap III). Aktivitas TGFβ1 bebas dalam ekstrak plasenta terakhir adalah setinggi 17 ± 8 pg/mg protein, sedangkan aktivitas TGFβ1 total adalah 242 ± 118 mg/protein. Hasil ini menunjukkan bahwa sebagian besar TGF1 terdapat dalam keadaan laten dan bahwa untuk mendapatkan kadar TGFβ1 yang tinggi, ekstraksi sebaiknya dilakukan dalam keadaan dipanaskan 70°C selama 30 menit atau tidak dipanaskan.

Abstract

This study was carried out to investigate the free and total activities of TGFβ1 in placental extracts after boiling for 30 min not heated and heated at 70°C for 30 min. The results showed that : (1) The free TGFβ1 activity before boiling was 10 ± 7 - 22 ± 9 pg/mg protein, whereas the total TGFβ1 activity was 100 ± 81 - 240 ± 51 pg/mg protein (steps I and II of the extraction procedure). (2) The free TGFβ1 activity after boiling (step III) was not detected, but after acidification to eliminate latency the total TGFβ1 activity in the final placental extract was 165 ± 120 pg/mg protein. (3) In the unheated placental preparation, the free TGFβ1 activity was 95 ± 52 pg/mg protein, whereas the total TGFβ1 activity was 838 ± 240 pg/mg protein (step III). In the final placental extract the free TGFβ1 activity was 6 ± 2 pg/mg protein, whereas the total TGFβ1 activity was 55 ± 13 pg/mg protein. (4) In the placental preparation heated at 70°C, the free TGFβ1 activity was 12 ± 4 pg/mg protein, whereas the total TGFβ1 activity was 379 ± 238 pg/mg protein (step III). In the final placental extract the free TGFβ1 activity was 17 ± 8 pg/mg protein, whereas the total TGFβ1 activity was 242 ± 118 mg/protein. These results showed that most of TGFβ1 activity was in its latent form and that in order to obtain a high TGF1 activity the extraction procedure should be performed at 70°C or not heated.

Keywords : *placenta, TGFβ1 activity*

INTRODUCTION

Wounds are defined as a disruption in the integrity of the skin, including the epidermis and dermis.[1] The process of wound healing includes 1) Vascular phase in which vasoconstriction, release of platelet derived growth factor (PDGF) and chemotactic agents occur, followed by vasodilation and release of more factors and inflammatory cells in the wound area; 2) Inflammatory phase in which inflammatory cells accumulate in the wound, and release of cytokines and the local hormone-like factors occur. In this phase polymorphonuclear leucocytes and macrophages also play a role in phagocytizing the cellular debris and the releasing of angiogenesis factor; 3) Epidermal phase. In this phase epithelialization occurs and various factors (transforming growth factor, epidermal growth factors, etc) stimulate the healing of the wound.

Wound healing is influenced by several factors, i.e. metabolic factors, and the microenvironment and wound dressing. Among the many factors in wound microenvironment, the role of growth factors is innegligible. Transforming growth factor β (TGFβ) has been proven as one of the most prominent growth

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factors released in the wound to promote the healing process.[2] TGFβ1, one member of TGFβ family, has been shown to be a potent growth factor that can stimulate fibroblast proliferation and acts as a chemoattractant agent for a variety of cells including neutrophils and macrophages.[3] The release of this peptide from platelets is recognized as essential to wound repair and according to some authors[4] it can accelerate wound healing.

Since placental tissue is riched in growth factors,[5] the placental extracts might be considered to be useful in accelerating tissue repair and promoting wound healing. The possible role of topical application of TGFβ and other growth factors in accelerating wound healing has been studied.[6] However, extraction procedure may influence the content and the integrity of growth factors.

Thus, in this study, we were interested in investigating the content of TGFβ1 in placental extracts and the influence of the extraction procedure on the content of TGFβ1.

METHOD

The samples of human placenta were obtained from a maternity clinic whose family was not interested to own them. After delivery, the placenta was rinsed and immediately preserved in a refrigerator.

The homogenization procedure to obtain 6 placental tissue fractions was carried out as follows:
To 1 kg of placenta tissue that had been trimmed off of connective tissue was added 20 liters of distillated water and the placentae were blendor homogenized (designated as homogenate II). The placental homogenate was boiled up for 30 min (designated as homogenate III) and filtered bymeans of a fine cloth (200 μm) (designated as homogenate IV). To the filtered homogenate was added 200 g of NaCl and mixed until thoroughly mixed. Then 10 ml of 10% HCl was further added and mixed until the homogenate reached a temperature of 40°C and pH 5 - 6 (designated as homogenate V). Sodium chloride was used to maintain the isotonicity of the homogenate and hydrochloric acid was added as a preservative. Finally, 100 g of phenol was added to the homogenate as a desinfectant agent. The addition of hydrochloric acid also served to provide an optimal condition for phenol to work. This fraction was filtered through a Whatman filter (designated as homogenate VI). Homogenate I was not a fraction in the routine procedure. It was obtained after homogenization of some crude placental tissue (and connective with it) in physiologic saline (1 : 1 w/w). The samples for protein and TGFβ1 activity measurements were obtained by withdrawing 0.5 ml of the homogenate from each step of the homogenization procedure.

TGFβ1 protein is quite stable in higher temperature, with the maximum temperature tolerated at about 80°C.[10] Based on this, we decided to determine the TGFβ1 activity in the unheated placental homogenates and in the placental homogenates heated at 70°C for 30 min, as well.

The activity of TGβ1 in the placental homogenates was determined by ELISA method according to the procedure described[7] with and without acidification. This acidification procedure is an in vitro method conversing the latent form to the active form of TGFβ1. In this protocol, a sample of placental homogenate was diluted 5 times with phosphate buffer containing 2.68 mM KCl, 136.89 mM NaCl, 1.47 mM KH_2PO_4, 8.10 mM NaH_2PO_4, 0.90 mM $CaCl_2$. $2H_2O$ and 0.49 mM $MgCl_2$. $6 H_2O$. In each 50 μl of the diluted sample was added 1 μl 1 N HCl. The mixture was incubated, then neutralized to pH 7.6 with 1 N NaOH. The activity of TGFβ1 was expressed as pg/mg protein. The protein content of the placental homogenates was determined according to Peterson,[8] using albumin as standards. Data are presented as mean ± SD of 3 or 4 repetitive experiments.

RESULTS

The results of the experiments are depicted in Tables 1, 2, and 3.
Data are presented from samples which had been treated and not treated with acid. From Table 1 it can be seen that the TGFβ1 can be detected in high concentrations in the placental homogenates which have not been boiled (from stage I & II). The boiling process in step III is associated with dissapearance of TGFβ1 both without or with acidification, whereas it reapeared in the subsequent process.

The activity of the enzyme is even higher after acidification procedure, indicating that most of the TGFβ1 in the placental homogenates is in latent form. The final fraction (from stage VI) shows activity only after acidification, suggesting that not all the TGFβ1 protein was denatured during the boiling process (stage III).

Table 1. The activity of TGFβ1 in placental homogenates with boiling for 30 min at stage III. Data are presented as means ± SD in pg/mg protein from 3 experiments.

Homogenate	TGFβ1 activity ± SD (pg/mg protein)	
	non acidified	acidified
I	15 ± 11	240 ± 51
II	10 ± 7	116 ± 29
III	-	-
IV	-	16 ± 8
V	-	2 ± 1
VI	-	165 ± 120

Table 2. The activity of TGFβ1 in placental homogenates without boiling. Data are presented as means ± SD in pg/mg protein from 4 experiments.

Homogenate	TGFβ1 activity ± SD (pg/mg protein)	
	non acidified	acidified
I	12 ± 8	100 ± 81
II	15 ± 10	174 ± 142
III	95 ± 52	838 ± 240
IV	47 ± 24	440 ± 203
V	39 ± 34	233 ± 75
VI	6 ± 2	55 ± 13

Table 3. The activity of TGFβ1 in placental homogenates which was heated at 70°C for 30 min at stage III. Data are presented as means ± SD in pg/mg protein from 3 experiments.

Homogenate	TGFβ1 activity ± SD (pg/mg protein)	
	non acidified	acidified
I	18 ± 10	162 ± 109
II	22 ± 9	220 ± 168
III	12 ± 4	379 ± 238
IV	20 ± 9	430 ± 168
V	300 ± 201	529 ± 359
VI	17 ± 8	242 ± 118

Tables 2 and 3 represent the activity of unheated placental homogenates and placental homogenates heated at 70°C, respectively. From the Tables it can be seen that the TGFβ1 activity presents in the fractions whether the placental tissues was not heated or heated until 70°C for 30 min at stage III and the acidification process consistently increased the activity of TGFβ1.

The activity of TGFβ1 in homogenates IV and V before and after acidification procedure (Tables 2 and 3) was higher than that in the final preparation (homogenate VI). The reason for this is unclear. So far there is no data available on the effect of phenol in the isolation of TGFβ1. These results suggest that the addition of phenol as a desinfectant agent should be reconsidered or substituted in the homogenization procedure.

DISCUSSION

This study demonstrates that placental extracts contain considerable activity of TGFβ1 and this activity is influenced by extraction procedure. The dissapearance of TGFβ1 activity after boiling process indicates that this process has resulted in denaturation of most of this growth factor.

Upon acidification procedure, the activity of TGFβ1 in placental homogenates is increased approximately 10 times as compared to the latent or unacidified preparations. This is about 10 times lower than the total TGFβ content found by Frolik et al.[9] Unfortunately since there is no data available in TGFβ1 content in placental homogenates, we cannot make any comparison with results from other authors.

TGFβ belongs to a family of related proteins which show amino acid sequence homology; other members of the family are inhibins, activins, Mullerian inhibiting substance and the product of the decapentaplegic complex of Drosophila.[10] There are 3 forms of TGFβ recognized at present, i.e. TGFβ1, TGFβ2, and TGFβ3. The rich source of TGFβ1 is platelet. However, TGFβ1 has also been isolated from placenta[5,9] and bovine kidney.[11] TGFβ1 has been localized by Graham et al[12] in the cytoplasm of first trimester and term syncytiotrophoblast, cytotrophoblast and decidual cells. It is thought that the TGFβ plays a role in the establishment and maintenance of placental function. TGFβ1 isolated from human platelets revealed that the complex consisted of 3 components with molecular weight of 13, 40, and 125-160 kDa.

The biological activity of TGFβ is highly cell type- and context- dependent. It inhibits the growth of many cells such as lymphocytes, epithelial cells and several types of malignant cells. However, TGFβ is a growth stimulator for mesenchymal cells, and accelerates experimental wound healing in rats.[3,4] Single application of TGFβ has been found to accelerate healing in gastric wounds in rabbits and to reverse glucocorticoid-induced wound healing deficiency in rats. In vivo, TGFβ has been found to stimulate fibrosis, angiogenesis, and wound healing.[4]

TGFβ accelerated the accumulation of collagen and DNA in wound chambers in rat and it appeared to be the most potent single factor in reversing doxorubicin-induced healing impairment in a wound chamber model.[13] However, combinations of TGFβ, platelet derived growth factor (PDG-F), epidermal growth factor (EGF) and insulin are more effective than any individual factor.

The mechanism of stimulation by TGFβ of fibroblast proliferation is unclear. It has been suggested that TGFβ enhances wound healing by direct stimulation of the synthesis of connective tissue by fibroblast and indirect stimulation of fibroblast proliferation mediated by PDGF.[14] $TGF\beta_1$ is also a potent chemoattractant for monocytes and fibroblasts; the growth factor may attract these cells to the site of inflammation and repair.[15]

Three different surface receptors have been identified for TGFβ, i.e. type I (65 kDa), type II (85-95 kDa), and type III (250-350 kDa), which may reflect different functional signalling mechanisms.[15]

$TGF\beta_1$ occurs mostly in latent form (L-$TBF\beta_1$).[10] L-$TGF\beta_1$ can be activated by certain treatments such as exposure to extreme pH values, Sodium dodecyl sulphate (SDS), and high concentration of urea. The NH_2-terminal part of the precursor molecules was probably responsible for the latency of the complex. In vivo the conversion of L- $TGF\beta_1$ into the active form is catalyzed by proteolytic enzymes present in the wound.

In this study we confirm the results from other authors,[9] that TGFβ presents in placental tissue. The presence of TGFβ and other growth factors may support the potent wound healing effect of the placental extract.[6] However, since the extraction of the placental tissue is carried out through several steps including boiling, this process might have lowered the content of TGFβ in the preparation, although in few experiments some $TGF\beta_1$ activity remained in the final fraction.

In accordance with this, we suggest that the extraction procedure of the placental tissue should be carried out after heating at 70°C for 30 min or without heating and that the addition of phenol should be reconsidered. These protocols, however, must be examined carefully by putting into account all the necessary measures that the final fraction obtained is germ-free.

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