



Broccoli Flower Extract (*Brassica oleracea L. var.italica* Plenck) Inhibits Photoaging by Increasing Type I Procollagen Expression in Human Skin Fibroblast

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Abstract: Photoaging of the skin is caused by intrinsic process superimposed with degenerative changes due to environmental sources such as ultraviolet irradiation. Ultraviolet B (UVB) reduces type I procollagen level and increases matrix metalloproteinase-1 (MMP-1) level in human skin which plays a major role in the process of photoaging. Broccoli flower extract (BFE) is a cruciferae group of vegetables which has multiple antioxidants. It had been studied, acting as MMP-1 inhibitor agent both at mRNA and protein level on skin photoaging in vitro. BFE were investigated for their capacity to regulate type I procollagen expression at protein level in primary human fibroblast culture irradiated by UVB 50 mJ/cm² and 100 mJ/cm². Type I procollagen protein expression was quantified by Enzyme Immuno Assay. The result of studies showed pre-treatment with various concentration of BFE increase type I procollagen expression. There were significant differences of the mean value of type I procollagen expression based on irradiation dose (p<0.05) and BFE concentration (p<0.05). There was also interaction between irradiation dose and BFE concentration (p<0.05). There was positive correlation between BFE concentration with type I procollagen expression. Therefore BFE has been proved to increase type I procollagen expression at protein level on UVB irradiated human skin fibroblast. BFE has a potential effect as antiphoaging agent in the near future.

Introduction

The skin aging process can be divided into intrinsic aging and extrinsic aging. Ultraviolet (UV) irradiation cause premature aging which is called as photoaging. It is caused by intrinsic processes superimposed with degenerative changes to solar radiation.^{1,2,3} UV irradiation disrupts the skin collagen matrix by stimulating collagen degradation by matrix metalloproteinase (MMP) and inhibiting procollagen production. The balance of MMP-1 and type I procollagen expression play an important role in photoaged skin. Alterations and deficiencies of collagen have been suggested to be a cause of the skin wrinkling observed in photoaged and naturally aged skin.⁴ Collagen degradation is mainly regulated by MMPs, while collagen synthesis is mediated by both transcriptional (gene/mRNA level) and post translational (protein level) processes.⁵ Broccoli (*Brassica oleracea L. var.italica* Plenck) is a cruciferous vegetables which has a great amount of antioxidant. It has been known that it consists of sulphoraphane, indole, beta carotene, quercetine, kaempferol, glutathione, vitamin A, C, E, selenium.^{6,7} In dermatology it has been proved as skin antinflammatory in UV- induced erythema⁸ and antimutagenic on epidermolysis bullosa.⁹ In previous studies we found that broccoli flower extract(BFE) down regulated MMP-1 mRNA expression and MMP-1 protein expression on UVB irradiated human fibroblast cell culture.¹⁰

Experimental

Material and methods

Plant material

Broccoli flowers were collected from broccoli field at Berastagi, North Sumatera, Indonesia. After processed to a standardized simplicia it was extracted with ethanol 96% in the laboratory of Faculty of Pharmacy University of Sumatera Utara, Indonesia.

Cell culture

The normal human fibroblast cells were aseptically isolated from preputium circumcised skin of healthy volunteer age 11-12 years old. After the epidermis and dermis were separated mechanically, the dermis was minced and attached on the surface of tissue culture flask. The cells were grown in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (GIBCO, Grand Island, NY USA). After 3 passages the fibroblast were used for the experiment.

Ultraviolet irradiation

The UV light source originated from a Philips TL 20 W(12 RS fluorescent sun lamp with an emission spectrum of 285-350 nm (peak at 310-315 nm). The cells were then exposed to 50 and 100 mJ/cm² dose of UVB light.

Treatment with BFE

BFE was dissolved in DMEM. The BFE concentration that use for treatment comprise of 25, 50 and 100 mg/ml. For treatment, the cells were maintained in culture media without FBS overnight, followed by treatment with BFE for 24 hours. The cells were rinsed twice with phosphate buffer saline (PBS) and UVB irradiation exposure were performed under a thin layer of PBS. Immediately after irradiation, the cells were incubated in serum-free fresh culture media containing BFE.

Enzyme Immuno Assay (EIA)

The supernatant from the cell culture was collected and the procollagen type I protein expression was quantified by procollagen type I C peptide EIA kit (Takara Biomedical Inc. Japan) at 450 nm using a microplate reader.

Statistical Analysis

Data were expressed as the mean value and with Fisher's least significant difference (LSD) were analyzed by analysis of variance (Anova) 2 ways, continued by multiple comparison test. Statistical significance was set a prior at $p < 0.05$. The correlation between BFE concentration and type I procollagen protein expression were analyzed by Spearman's correlation test.

Result

Effect of BFE on type I procollagen protein expression

To establish effect of BFE on type I procollagen protein expression in photoaging induced by UVB radiation, EIA analysis were performed. It revealed that the BFE up regulated UVB –induced reduction of type I procollagen expression (Fig.1). Anova two ways showed there were significant differences of mean value of type I procollagen protein expression between every group based on irradiation dose ($p < 0,05$) and BFE concentration ($p < 0,05$) (Table 1). Continued by multiple comparison test (LSD) showed significant differences ($p < 0,05$) between every group based on irradiation dose and BFE concentration. These result indicated that BFE increased of type I procollagen expression at the protein level in UVB-irradiated human fibroblast.

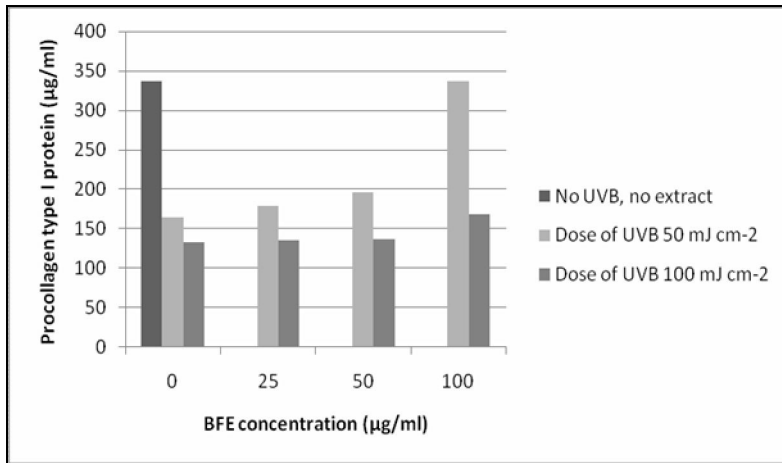


Figure 1. Effect of BFE on type I procollagen protein expression

Table 1. Mean value of type I procollagen protein expression between every group based on irradiation dose and BFE concentration.

Sources	Sum of squares	Df	Mean square	F	p
Irradiation dose	34094,525	1	34094,525	1×10^{10}	0,0001*
Extract concentration	41852,762	3	13950,921	6×10^9	0,0001*
Interaction dose * concentration	17483,594	3	5827,865	2×10^9	0,0001*
Error	$4,20 \times 10^{-5}$	18	$2,33 \times 10^{-6}$		0,0001*
Total	1220951,621	27			0,0001*
Corrected Total	157603,336	26			

Correlation between BFE concentration with type I procollagen protein expression

By Spearman’s correlation test we found there were significant positive correlation between BFE concentration with type I procollagen protein expression at 50 mJ/cm² UVB irradiation dose (r=0.975, p<0.01) and at 100 mJ/cm² UVB irradiation dose (r=0.973, p<0.01) (Table 2).

Table 2. Correlation between BFE concentration with type I procollagen protein expression.

		n	r	p
Extract concentration				
	Procollagen type I protein (50)	12	0,975	0,0001
	Procollagen type I protein (100)	12	0,973	0,0001

Spearman’s correlation test; Correlation is significant at the 0,01 level (2-tailed); 50: UVB irradiation dose 50 mJ cm-2; 100: UVB irradiation dose 100 mJ cm-2; r: correlation coefficient.

Discussion

Skin ages with time progress. Both intrinsic aging and photoaging process involve the expression and degradation of several molecules participating in the metabolism of the connective tissue, including changes in the amount of extracellular matrix components such as collagen and in MMPs activities.^{11,12} The balance of collagen synthesis and degradation is crucial to form wrinkle of photoaging.⁵ UVB irradiation has been demonstrated to produce reactive oxygen species (ROS) in the cells and skin which induced MMPs that degrade collagen.³ Collagen fiber is primarily synthesized by fibroblast as procollagen protein, which is secreted and

further processed to be a collagen fiber in the extracellular matrix.¹³ Photoaging is a process involving alteration of type I collagen which is the major component of the dermis. Among collagen, type I is the most abundant and comprises between 85% of the total collagen in the skin. Type I collagen degradation is the major cause of wrinkle formation.¹⁴ The use of antioxidant is an effective approach to prevent symptoms related to photo-induced aging of the skin. Strategies to prevent or at least minimize ROS-induced photoaging necessarily include protection against UV irradiation and antioxidant homeostasis.¹⁵

Broccoli flower extract (*Brassica oleracea var. italica* Plenck) is an antioxidant plant which has been studied in dermatology. Sulforaphane, the most potent antioxidant in broccoli, has antiinflammatory effect which is shown by preventing erythema from sunlight.⁸ In our previous study, we reported that BFE decreased the level of MMP-1 expression both at mRNA and protein level in the process of photoaging induced by UVB irradiated in vitro.¹⁰ Therefore on the other hand we investigated whether BFE also may increase the level of type I procollagen expression. Our results showed that BFE has enhancer activity on type I procollagen protein expression in UVB irradiated human skin fibroblast. We also found there were interaction between UVB irradiation dose and BFE concentration to the expression of type I procollagen. This result indicated that both factors together influence the mean value of type I procollagen expression. We also found there were positive correlation between BFE concentration with type I procollagen protein expression, thereby the increasing of BFE concentration leads the type I procollagen expression increased. These results demonstrates the inducer effect of BFE on type I procollagen expression via protein assay in skin photoaging process in vitro. Therefore it is suggested that BFE should be viewed as a potential therapeutic agent for preventing and treating skin photoaging based on decrease the MMP-1 expression and increase procollagen type I expression.

Conclusion

BFE had inhibitory effect on skin photoaging induced by UVB in vitro by increasing type I procollagen expression at protein level in human skin fibroblast culture. It is suggested further studies on BFE involving human subjects should be carried out in the near future.

Acknowledgement

Author thanks to Prof.Dr.dr Hardyanto Soebono, Sp.KK(K), Prof.Drs. Sumadio Hadisahputra, Apt., Ph.D, dr. Adang Bachtiar, MPH, D.Sc, Dr.dr. Imam Budi Putra, MHA, SpKK for support and cooperation. The author have no funding or support to report. No conflict of interest.

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