The Effect of Capsaicin and The Expression of Psoriasin Protein on Human Keratinocyte HaCaT Cell Line *in vitro*

ผลของแคปไซซินและการแสดงออกของโปรตีนโซไรซินในเซลล์สร้างเคอราทินในหลอดทดลอง

Nichakarn Kwankeaw (ณิชกานต์ ขวัญแก้ว)* Dr.Netnaphis Warnnissorn (ดร.เนตรนภิส วรรณิสสร)**
Dr.Uthai Wichai (ดร.อุทัย วิชัย)*** Dr.Prateep Warnnissorn (ดร.ประทีป วรรณิสสร)****

ABSTRACT

Capsaicin (8-Methyl-N-vanillyl-6-nonenamide) is found in hot peppers with the molecular formula of $C_{18}H_{27}NO_3$. Capsaicin has been reported to relieve pain, inflammation and psoriasis, which is an autoinflammatory skin disease. The cause of psoriasis is not fully understood, but it is expected to be an abnormally excessive and rapid growth of the keratinocyte cell. Psoriasin (S100A7), a member of the S100 protein family, is highly expressed in hyper-proliferative skin conditions. Our objective was to study the effect of capsaicin on human keratinocyte HaCaT cell line *in vitro*. We assessed the cell viability using the WST-1 assay kit. Capsaicin decreased the survival rate of HaCaT at concentrations above 200 µM (*$p<0.05$), by flow cytometry we detected intracellular staining of psoriasin in untreated HaCaT cell line. We found psoriasin protein in untreated HaCaT cells. Thus, the results of this study can be used as a guideline for further *in vitro* studies of psoriasis model

บทคัดย่อ

แคปไซซิน (8-Methyl-N-vanillyl-6-nonenamide) คือสารสำคัญที่ให้ความเผ็ดร้อนในพริก มีสูตรโมเลกุล $C_{18}H_{27}NO_3$ จากการศึกษาพบว่าแคปไซซินสามารถบรรเทาอาการปวด ต้านการอักเสบ การศึกษาแคปไซซินในสะเก็ดเงินซึ่งก็เป็นโรคที่เกี่ยวข้องกับการอักเสบเรื้อรังของผิวหนัง สาเหตุของการเกิดสะเก็ดเงินยังไม่เป็นที่ทราบแน่ชัด แต่คาดว่าเกิดจากการแบ่งและผลัดเซลล์ผิวหนังที่เร็วผิดปกติ นอกจากนี้พบว่ามีการแสดงออกของโปรตีนโซเรซิน (psoriasin/S100A7) ในโรคทางผิวหนังที่เกี่ยวข้องกับความผิดปกติในการแบ่งตัวเพิ่มจำนวนของเซลล์สร้างเคอราทิน จึงนำมาสู่วัตถุประสงค์ของงานวิจัยเพื่อศึกษาผลของแคปไซซินต่อเซลล์สร้างเคอราทิน HaCaT ในหลอดทดลอง จากการทดลองวัดอัตราการรอดชีวิตของเซลล์สร้างเคอราทินด้วยวิธี WST-1 พบว่าแคปไซซินที่มากกว่า 200 ไมโครโมลาร์ (*$p<0.05$) มีผลต่ออัตราการรอดชีวิตของสร้างเคอราทิน HaCaT นอกจากนี้ได้ทำการวัดปริมาณการแสดงออกของโปรตีนโซไรซินด้วยเทคนิค Flow cytometer พบว่ามีการแสดงออกของโปรตีนโซไรซินในเซลล์สร้างเคอราทิน HaCaT ที่ไม่ได้รับการกระตุ้น จากผลการทดลองทั้งหมดนี้จะนำไปเป็นแนวทางศึกษาต่อในแบบจำลองโรคสะเก็ดเงิน

Keywords: Capsaicin, Psoriasis, Psoriasin/S100A7 protein
คำสำคัญ: แคปไซซิน สะเก็ดเงิน โปรตีนโซเรซิน

* *Student, Master of Science Program in Biochemistry, Faculty of Medical Science, Naresuan University*
** *Lecturer, Department of Biochemistry, Faculty of Medical Science, Naresuan University*
*** *Lecturer, Department of Chemistry, Faculty of Science, Naresuan University*
**** *Assistant Professor, Department of Medicine, Faculty of Medicine, Naresuan University*

Introduction

Psoriasis is a chronic inflammatory skin disease that affects approximately 1-3 % of the world's population (Parisi et al., 2013). It is characterized by hyperproliferation of keratinocytes, dilation and growth of dermal. Interestingly, S100A7 or psoriasin is overexpressed in hyperproliferative skin disease, in which it is believed not only exhibiting antimicrobial function, but also inducing immunomodulatory activities, including chemotaxis and cytokine/chemokine produce (Hattori et al., 2014), which as the clinical features of psoriasis. There are several methods of psoriasis treatments depending on the severity of the rash. In general, the severity of the disease is divided into three levels: mild, moderate, and severe. Approximately 80 % of patients with psoriasis are of less common types, but can be treated with topical drug. Topical steroid are usually the first line of defense in treating psoriasis, with potential side effects including skin damage such as skin thinning, changes in pigmentation, easy bruising, stretch marks, redness and dilated surface blood vessels (Schäcke et al., 2002). In recent years, topical calcipotriol is a widely used treatment for psoriasis worldwide and has been shown to improve psoriatic plaques (Ito et al., 2016). Calcipotriol has fewer side effects than topical steroids, but it is more expensive.

Calcipotriol is a vitamin D analog that inhibits keratinocyte proliferation, controls keratinocyte differentiation, and inhibits the secretion of cytokines that stimulate the inflammatory leukocytes (Scott et al., 2001). *In vitro* studies have shown that vitamin D3 analogues decreased the proliferation and induced differentiation of keratinocytes and had strong immunomodulating effects (Takahashi et al., 2003). Increased epidermal Bcl-2 expression after calcipotriol therapy may be attributed to the restoration of normal basal cell activities because Bcl-2 expression may control the increased apoptosis induced by the treatment (El-Domyati et al., 2007).

Capsaicin (8-Methyl-N-vanillyl-6-nonenamide) is found in hot peppers with the molecular formula of $C_{18}H_{27}NO_3$. Capsaicin has been reported to relieve pain, inflammation and psoriasis (Gupta et al., 2016). Many studies have demonstrated that capsaicin can inhibit several cancers such as prostate, lung, and leukemia. Capsaicin is an irritant material. Dermal exposure to this capsaicin results in burning or stinging pain in humans.

Objectives of the study

Based on the previous findings, the aim of this research was to study the effect of capsaicin and the expression of psoriasin protein on human keratinocyte HaCaT cell line *in vitro*.

Methodology

Preparation of Capsaicin

Capsaicin of 97 % purity (MW= 305.41 g/mol; obtained from Uthai Wichai, Ph.D., Department of Chemistry, Faculty of Science, Naresuan University. The stock solution of capsaicin was prepared by dissolving capsaicin in 0.1 % DMSO and dilutions were prepared using Dulbecco's Modified Eagle's Medium (DMEM).

Chemicals and Cell culture

The chemical, 4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1, 3-benzene disulfonate (WST-1) was from Takara Bio USA, Dimethyl sulfoxide (DMSO) was from Merck KGaA, Germany, DMEM and Fetal bovine serums (FBS) were from U.S. Hyclone™. Human Skin keratinocyte (HaCaT) cell were obtained from CLS Cell Lines Service GmbH, Eppelheim, Germany and cultured in DMEM supplemented with 10% FBS in 5% CO_2 atmosphere at 37 °C. Every second day, the medium in the culture dishes was replaced with fresh culture medium to allow the growth of HaCaT cells. When the cells reached confluent, they were harvested using 0.05% trypsin-EDTA (Gibco, Grand Island, NY, USA) and fresh culture medium was added to produce a single cell suspension.

Cell proliferation and viability assay using HaCaT cells

HaCaT cells at a cell density of $1x10^5$ cell/ml were seeded into a 96-well microplate containing DMEM supplemented with 10 % FBS and incubated in an incubator of atmosphere of 5 % CO_2 and 37 °C. After 24 h incubation, the culture medium was replaced with fresh DMEM medium. Capsaicin in DMSO was added in the wells containing culture to a final concentration of 5-500 µM. Cells with DMEM medium or DMSO alone was maintained to check for possible cytotoxicity of the solvent. Cells without added compound were used as a negative control. After incubation at 37 °C in a 5% CO_2 atmosphere, the cells treated with different concentration of capsaicin were added with 100 µl of fresh medium along with 10 µl of WST-1 solution and further incubated at 37 °C for 4 h. After performed the proliferation assay, absorbance of each sample was measured at 450 nm (Microplate Reader BIO-RAD iMark™). All experiments were performed in triplicate.

Detected psoriasin (S100A7) in HaCaT cell line (unstimulated) by Flow cytometry

HaCaT were growth in DMEM culture medium containing 10 % fetal bovine serum in 6-well plate at 37 °C in 5 % CO_2. After 24 h when the cells reached confluent growth, they were harvested using 0.05% trypsin-EDTA. After centrifugation, pellets of floating and adherent cells were washed with 1x phosphate buffered saline pH 7.4 (PBS), fixed in 1% paraformaldehyde for 15 min. After two washes with 0.5% FBS in PBS, cells were incubated for 30 min on ice in permeabilized agents (PBS containing 0.5% FBA and 0.2% Tween20). After two washes with 0.5% FBS and 0.2% Tween20 in PBS, the cells were incubated with Mouse / IgG1, kappa S100A7 antibody (Pierce™ Thermo Scientific) incubated for 1 h on ice at room temperature, Then the cells were twice rinsed with PBS containing 0.2% Tween20 0.5% FBS and stained with FITC rat anti-mouse IgG1 (BD Pharmingen™) for 30 min in the dark at room temperature. Detected psoriasin (S100A7) in HaCaT cells was analyzed by FACS Caliber flow cytometer (BD BectonDickinson). Quantitative analysis of the FACS data was done by using BD CellQuest Pro software.

Statistical methods

All experiments were performed at least three repeats. Statistical analysis was performed using ANCOVA (Analysis of Co-variance) which, used analyses a between group and within groups. Values were presented as mean ± S.D differences were considered significant at $*P < 0.05$ statistical significance.

Results

Cytotoxic effects of capsaicin on the viability of HaCaT cell line

After 24 h treatment, capsaicin significantly decreased the survival rate of HaCaT at concentrations of 200 µM and above (*p<0.05) (Fig.1). No cytotoxic effect was observed in the culture cells treated with DMSO.

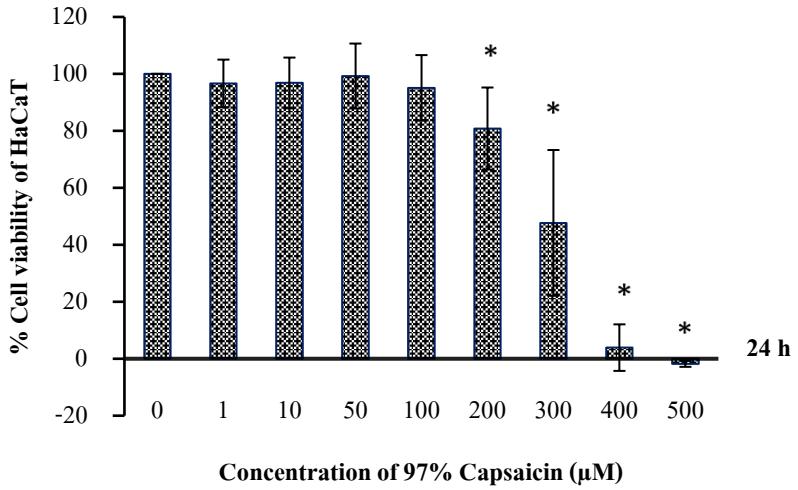

Figure 1 Percentage cell viability of HaCaT cells after 24 h incubation with the various concentration of capsaicin at 37°C in 5% CO2 atmosphere. Each value was shown is the mean ±SD. *p<0.05 compared with the control.

Psoriasin (S100A7) expression in unstimulated HaCaT cells

The intracellular staining unstimulated HaCaT cells with S100A7/ psoriasin was detected compared to isotype control (Fig.2) by flow cytometry

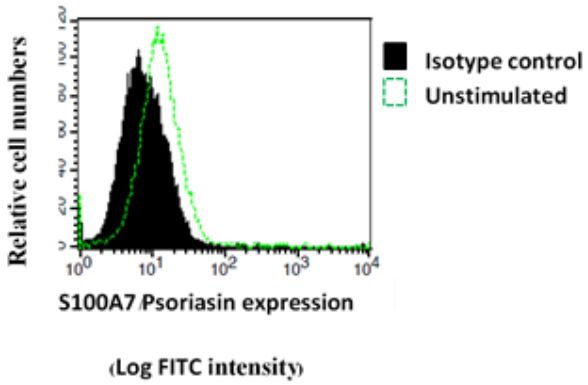

Figure 2 Unstimulated HaCaT cell line intracellular expression of S1007A/Psoriasin (Green line) compared with the Isotype controls (black) by flow cytometry.

Discussion and Conclusions

Psoriasis is a common chronic, inflammatory, hyperproliferative skin disease. In the past, psoriasis is believed to be a disease caused by abnormal in the process controling the proliferation of skin cells for unknown reasons. However, nowadays it is accepting that immune cells play an important role in the proliferation of keratinocytes in psoriasis patients. In recent studies, natural products such as rhodomytone has immunomodulatory effects on innate immune responses (Chorachoo et al., 2016) and exhibit an antiproliferation.

Capsaicin was previously shown to reduce inflammation, antiproliferation of cells, and reduced side effects to human. In recent years, increasing evidence has demonstrated that a number of natural products, especially from plants has been used extensively. Based on the previous findings, alternative treatments that can reduce the use of imported drugs many reduce the cost of healthcare.

Capsaicin showed cytotoxicity on HaCaT cells by significantly decrease the survival rate of HaCaT cells in a dose dependent manner. Cytotoxicity of capsaicin is an important criteria for choosing an effective anti-psoriasis drug for psoriasis patients.

Recent studies have demonstrated that S100A7/psoriasin was overexpressed in lesions of psoriatic skin and was considered to play a role in inflammation processes by regulating the differentiation of keratinocyte (Anderson et al., 2009; Hattori et al., 2014). In addition, the expression of psoriasin on HaCaT cells without immunotropic cytokine stimulation were found in our study.

In conclusion, our data has provided insights into the cytotoxic effects of capsaicin and the expression of psoriasin protein on human keratinocyte HaCaT cell line. Thus, the results of this study may provide as a guideline for further studies on *in vitro* psoriasis model.

Acknowledgements

This work was supported by Naresuan University Research Fund.

References

Anderson KS, Wong J Fau - Polyak K, Polyak K Fau - Aronzon D, Aronzon D Fau - Enerback C, Enerback C. Detection of psoriasin/S100A7 in the sera of patients with psoriasis. Br J Dermatol 2009; 160(2): 325-32.

Chorachoo J, Saeloh D, Srichana T, Amnuaikit T, Musthafa KS, Sretrirutchai S, et al. Rhodomyrtone as a potential anti-proliferative and apoptosis inducing agent in HaCaT keratinocyte cells. Eur J Pharmacol 2016; 772(5): 144-51.

El-Domyati M, Barakat M Fau - Abdel-Razek R, Abdel-Razek R Fau - El-Din Anbar T, El-Din Anbar T. Apoptosis, P53 and Bcl-2 expression in response to topical calcipotriol therapy for psoriasis. International journal of dermatology 2007; 46: 468-74.

Gupta R, Gupta M, Mangal S, Agrawal U, Vyas SP. Capsaicin-loaded vesicular systems designed for enhancing localized delivery for psoriasis therapy. Artif Cells Nanomed, Biotechnol 2016; 44(3): 825-34.

Hattori F, Kiatsurayanon C, Okumura K, Ogawa H, Ikeda S, Okamoto K, et al. The antimicrobial protein S100A7/psoriasin enhances the expression of keratinocyte differentiation markers and strengthens the skin's tight junction barrier. British Journal of Dermatology 2014; 171(4): 742-53.

Ito K, Koga M, Shibayama Y, Tatematsu S, Nakayama J, Imafuku S. Proactive treatment with calcipotriol reduces recurrence of plaque psoriasis. Journal of Dermatology 2015; 43: 402-5.

Parisi R, Symmons DPM, Griffiths CEM, Ashcroft DM. Global Epidemiology of Psoriasis: A Systematic Review of Incidence and Prevalence. Journal of Investigative Dermatology 2013; 133(2): 377-85.

Schäcke H, Döcke W-D, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. Pharmacology & Therapeutics 2002; 96(1): 23-43.

Takahashi H, Ibe M, Kinouchi M, Ishida-Yamamoto A, Hashimoto Y, Iizuka H. Similarly potent action of 1,25-dihydroxyvitamin D3 and its analogues, tacalcitol, calcipotriol, and maxacalcitol on normal human keratinocyte proliferation and differentiation. Journal of dermatological science 2003; 31(1): 21-8.