원 저

cDNA microarray gene expression profiling of melittin and mast cell degranulation peptide in human mast cell strain

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Abstract

봉독의 주요성분인 Melittin과 MCDP이 비만세포주에서 유전자 발현에 미치는 영향에 대한 microarray 분석

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비만세포는 염증 및 알레르기 반응과 관련하여 우리 몸에서 주요한 작용을 하는 세포이다. 봉독은 현재까지 진통기전에 관련된 모델로 연구가 진행되어 왔으나, 최근에는 항염증이나 항알러지반응 등에서 면역세포와 관련한 연구가 진행 중에 있다. 본 연구는 봉독의 주요성분인 melittin과 MCD Peptide가 비만세포주에서의 유전자 발현에 미치는 영향을 연구함으로써 향후 유전자 연구에 관련한 기초를 제시하고자 하였다.

본 연구에서는 사람의 비만세포주를 이용하여, 세포독성 실험을 거쳐서 얻은 유효농도에서 각각 melittin과 MCD Peptide를 처치하고, 이때 변화하는 유전자의 발현양상을 microarray 분석기법을 통하여 정보를 얻었다.

실험적 통계에 의하여 global M이 1 또는 -1이상인 것을 유의한 것으로 보았을 때, melittin에서는 모두 7개의 유전자가 항진되고, 8개의 유전자가 억제되었다. MCDP에서는 7개의 유전자가 항진되고 17개의 유전자가 억제되었다. 이들 유전자들이 주로 관련하는 체내의 작용은 세포내에서 단백결합, lymphocyte 기능의 활성화, macrophage 항원관련 및 세포핵의 수용체, GABA A receptor 관련물질, cAMP 반응요소와 연관된 단백질, 보체계 8번 및 B-cell 관련물질, 다낭성 신질환에 관련된 단백물질, 염증관련물질, 혈액응고에 영향을 주는 단백물질등과 연관이 되었다.

이러한 분석결과를 통하여 동복에서의 주요약리작용을 담당하는 melittin과 MCD peptide의 작용기전을 밝히는데 보다 유용한 자료를 얻을 수 있었으며, 향후에 봉독의 주요성분 및 전체 봉독액이 항알레르기반응이나 항염증작용에 미치는 영향에 대한 심도있는 연구가 필요할 것으로 사료된다.

I. Introduction

Although bee venom (BV) has been clinically used to relieve pain and reduce inflammation[1-3] in traditional Oriental medicine, especially in chronic inflammatory diseases such as rheumatoid arthritis (RA), a variety of compounds in bee venom may increase the risk of allergic reaction, and a possible contamination of bacteria in non-sterilized bee venom may cause infection. BV has both inflammatory and anti-inflammatory activity. The major constituent responsible for the controversies by BV is not apparent.

BV contains several biologically active non-peptide substances as well as 2 major known peptides, the hemolytic peptide melittin and the neurotoxic peptide apamin, and a number of minor peptides. The other peptides in bee venom, unlike mast cell degranulating (MCD) peptide, have no known anti-inflammatory properties[4].

Melittin, which is a principal protein of honeybee venom, can be a substitution in humans as a single component may reduce the risk of allergic reactions, and sterilized melittin may avoid infection[5].

Mast cells are the main target cells for immunoglobulin E (IgE) molecules which bind with high affinity to FcεRI receptors on the mast cell surface. When receptor-bound IgE molecules are cross-linked by multivalent allergens, the receptors are activated and cause the mast cell to degranulate and release histamine and other inflammatory mediators[6]. MCD peptide is a natural compound found in BV and one of the strongest natural histamine secretagogues, stimulating mast cell degranulation at low concentration. At higher concentrations it has been found to inhibit mast cell degranulation[4].

cDNA microarray methods are applied to the study of gene expression, DNA sequence, novel genes and gene mutants, DNA polymorphism, and in screening drugs, diagnosing diseases and mapping gene library[7]. Microarray analysis is a technique which has been shown to be of particular utility in area including simultaneous profiling of global gene expression and uncovering new genes or new functions of known genes.

Studies involving gene expression following BV administration have been published. Microarray analysis of gene expression in a human mast cell line following BV treatment have been previously reported. However, there has yet been no report on the effect of melittin and MCD peptide, the major constituents of bee venom, in a human mast cell line.

In the present study, global gene expression profiling was carried out in an effort to understand the effect of melittin and MCD peptide on human mast cell strain.

II. Materials and Methods

1. Cells of the culture

The human mast cells lines derived from leukemia patients were cultured in Iscove's modified Dulbecco's medium (IMDM) fluid supplemented with 10% fetal bovie serum (FBS), 1% penicillin-streptomycin (Gibco BRL, USA). The cultured cells were incubated at 37℃ in a humidified cell incubator and an atmosphere consisting of 5% CO2-95% room air. The culture fluid was changed every 2 days.

2. Cytotoxicity assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide) assay was carried out as per the manufacturer's protocol (Roche, Germany) to investigate the effects of melittin and MCD peptide. The MTT assay is based on the cellular reduction of tetrazolium salts to their intensely colored formazans[8]. The MTT assay is conventionally used for measuring cell proliferation, however, because the metabolic conversion of the MTT salt is mediated by active mitochondrial dehydrogenase in living cells, the test can also be used to assess cell viability[9-10]. The formazan solubilized and spectrophoto metrically quantified using an ELISA reader (Bio-Tek, USA). 5×104 cells were cultured in each well of a 96-well culture plate with 100 ml of serum-free fluid and melittin (Sigma, USA) or MCD peptide (Sigma, USA) for 12 hr incubation. The concentrations of each group treated melittin were 10-3, 10-2, 10-1, 1, 10 mg/ml, and those of MCD peptide were 10-4, 10-3, 10-2, 10-1, 1 mg/ml. Non-treated group used as control. 10 ml MTT labeling reagent solution was added to each well, and cells were then incubated for another 4 hr. The purple formazan salts thus produced were solubilized by adding 100 ml solubilization solution to each well and incubating overnight, again at 37℃ in a humidified cell incubator and an atmosphere consisting of 5% CO2-95% room air. The solubilized solution was colorimetrically assayed using an ELISA reader at a wavelength of 595 nm, with a reference wavelength of 690 nm. Percent cell viability was calculated as the absorbance rate of the experimental group over that of the control group. Student's t-test was used for statistic analysis and p-value below 0.05 indicated statistical significance.

3. RNA isolation

Total cellular RNA was extracted using the Mini RNA Isolation ⅡTM (Zymo Research, USA). This method has been validated for simply and reliably isolating total RNA in 10 min and improving chemistry inactivates nucleus more efficiency than classic methods. Pellet the cells for 5 min by centrifugation at 300-500g. Removed the supernatant completely. Loosed the cell pellet by lightly vortex or flicking. Added 600 ml of ZR RNA Buffer (Zymo Research, USA). Lightly vortex to mix completely. Transfered the sample homogenized in the ZR RNA Buffer to a Zymo-Spin Ⅲ Column (Zymo Research, USA) and placed the column into a 2 ml collection tube. Spined at full speed in a microcentrifuge for 1 minute. Added 350 ml of RNA Wash Buffer to the Zymo-Spin Ⅲ Column and centrifuged at full speed for 1 min to wash. Added another 350 ml of RNA Wash Buffer and centrifuged at full speed for 1 min. Transfered the column to a new 1.5 ml tube. Added 50 ml RNase-free Water directly to the membrane of the Zymo-Spin Ⅲ column. Waited for 1 min. Spined briefly to elute RNA.

4. Dye labeling protocol

The labeling reaction master mix (total 300 ml : 5× reverse transcription buffer, 0.1M DTT, 100 nM dATP, dCTP, dGTP, dTTP, DEPC-treated DW) was made. Set up 2 reactions, Cy3 control RNA and Cy5 control RNA, mixed with oligo(dT). The control group was used with Cy3 dye (Amersham Phamacia, Sweden), and experiment groups with Cy5 dye (Amersham Phamacia, Sweden). Incubated the reaction mixture (RNasin, Labeling-reaction master mixture, CyX-dUTP, Super Script Ⅱ RT) at 70℃ for 10 min and chilled on ice for 1 min. Mixed thoroughly with gentle pipetting.

Incubated at 42℃ for 1hr. Added another 1 ml of SuperScript II RT into the labeling reactions and incubated for additional 1 hr. Spun down the reaction. Added 2 ml of 500 mM NaOH and 20 nM EDTA. Heated the reaction at 70℃ for 30 min to degrade the RNA. Neutralized the reaction with PCR clean-up kit as recommended by manufacturer. Calculated the pmole nucleotide, pmole Cy3, Cy5, and nucleotide/dye ratio. Dried the Cy3 and Cy5 sample in a SpeedVac and continued the hybridization process immediately.

5. Hybridization and washing

Before hybridization, incubate slide in prehybridization solution prewarmed to 42℃ in a slide box for one hour at 42℃. Washed the slide by dipping five times in DW. Dried the slide by centrifuge at 650 rpm for 5 min. The slide used immediately after prehybridization. Dissolved the dried labeled sample in a reasonable volume (220 ml) of hybridization buffer Heat the labeled sample preparation for 5 min at 95℃. Spinned down for 30 sec. Placed slide in the hybridization chamber. Put 20 ml of 3×SSC to the chamber at both ends of the slide. Pipette the labeled target onto the slide surface. Carefully placed the coverslip on the top of the slide. Tried to reduce the formation of air bubbles. Sealed the hybridization chamber and incubated in 42℃. Hybridization oven for more than 16 hr. Disassembled hybridization chamber right side up. Removed the coverslip by immersing the slide in 2×SSC/0.1% SDS at 42℃ (500 ml solution : 50 ml of 20×SSC, 5 ml of 10% SDS, 492.5 ml of DW). Placed the slide in 2×SSC/0.1% SDS for 5 min at 42℃. Placed the slide in 0.1×SSC/0.1% SDS for 10 min at RT (500 ml solution : 2.5 ml of 20×SSC, 5 ml of 10% SDS, 492.5 ml of DW). Placed slide in 0.1×SSC for 1min at RT (500 ml solution : 2.5 ml of 20×SSC, 492.5 ml of DW). Repeated 4 times. Dried slide by centrifuge at 650rpm for 5 min.

6. Scanning and image analysis

The hybridized microarray was scanned with a confocal laser scanning microscope (ScanArray 3.0, Packard Inc, USA) at 532 nm for Cy3 and 635 nm for Cy5. Image analysis using GenePix (Axon Inc, USA) produced quantitative values for each microarray spot. Pixel intensity of the background was subtracted from those of microarray spots. Spot intensities were normalized using the intensities generated by house-keeping genes. Normalized spot intensities were calculated into gene expression ratios between the control and treatment groups. Global M counts over 1 or −1 were considered to be of significance.

7. Data analysis

Quadruplicate RNA samples were obtained for chemical. cDNA microarray analysis was performed twice for each RNA sample. The fluorescent intensity of each spot was calculated by local median background subtraction. It was used the robust scatter-plot smoother LOWESS function to perform intensity dependent normalization for the gene expression. Scatter plot analysis was made by Microsoft Excel 2000 (Microsoft, Redmond, WA). Significance Analysis of Microarray (SAM) was performed for the selection of the genes with significant gene expression changes[16]. The statistical significance of the differential expression of any genes was assessed by computing a q-value (the lowest false discovery rate at which the gene is significant) for each gene. Genes were considered differentially expressed when

logarithmic gene expression ratios in three independent hybridizations were more than 1 or less than -1, i.e., a 2-fold difference in expression level, and when the p-values were < 0.1 .

Ⅲ. Results

1. Cytotoxicity assay

1) The viability of melittin treated human mast cells

Twelve hours incubation with melittin at the following concentration produced the respective percent viabilities relative to that of the control group in human mast cell line cultures; 93.6% with 10-2 mg/ml melittin, 86.7% with 10-1 mg/ml melittin, 86.2% with 1 mg/ml and 71.3% with 10 mg/ml melittin. Percent viability with 10-2, 10-1, 1, 10 mg/ml melittin groups were statistically significant compared with control group (Fig. 1). In this study, it was used the 1 mg/ml concentration
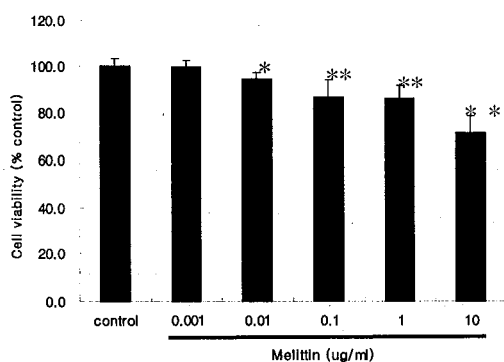

Fig. 1. Cell viability (%) of human mast cell after 12 hour incubation of melittin at the respective concentrations

Percent viabilities of the treatment groups were calculated by MTT colorimetric assay relative to that of the control group. Values were given as percent. Asterisk (*) indicates statistical difference from the control group.
*: p<0.05, **: p<0.01, ***: p<0.001

treated group.

2) The viability of MCD peptide treated human mast cells

Twelve hours incubation with MCD peptide at the following concentration produced the respective percent viabilities relative to that of the control group in human mast cell line cultures; 91.9% with 10-2 mg/ml MCD peptide, 83.4% with 10-1 mg/ml MCD peptide, 76.6% with 1 mg/ml MCD peptide. Percent viability with 10-2, 10-1, 1 mg/ml MCD peptide groups were statistically significant compared with control group (Fig. 2). In this study, it was used the 0.1 mg/ml concentration treated group .

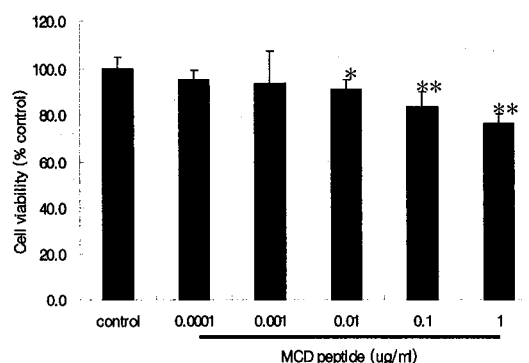## 2. Gene expression profiles of

Fig. 2. Cell viability (%) of human mast cells after 12 hour incubation of MCD peptide at the respective concentrations

Percent viabilities of the treatment groups were calculated by MTT colorimetric assay relative to that of the control group. Values were given as percent. Asterisk (*) indicates statistical difference from the control group.
*: p<0.01, **: p<0.001

melittin treated cells

In the TwinchipTM Human-8K cDNA microarray image of gene expression in human mast cells treated with melittin for 12 hours, we got the results. The primary data was

normalized by the total spots of intensity between two groups, and then normalized by the intensity ratio of reference genes such as housekeeping genes in both groups. The expression ratio of melittin-treated and human mast cell control was converted to log2 ratio.

Genes were screened out that exhibited different expressions in all chips, there were 7 up-regulated and 8 down-regulated genes in the gene expression profiles of melittin treated group (Table 1, 2).

Gene expression was profiled with TwinchipTM Human-8K cDNA microarray.

Cut-off level of two-fold change was used for the gene up-regulated by melittin treated.

Gene expression was profiled with TwinchipTM Human-8K cDNA microarray.

Cut-off level of two-fold change was used for the gene down-regulated by melittin treated.

3. Gene expression profiles of MCD peptide treated cell

In the TwinchipTM Human-8K cDNA microarray image of gene expression in human mast cells treated with MCD peptide for 12 hours, we got the results. The primary data was normalized by the total spots of intensity between two groups, and then normalized by the intensity ratio of reference genes such as housekeeping genes in both groups. The expression ratio of MCD peptide-treated and human mast cell control was converted to log2 ratio.

Genes were screened out that exhibited

Table 1. Genes Showing Melittin-induced Up-regulation in Human Mast Cells

Gene	Chromosome	Title	Global M
ZPBP	7p14.3	zona pellucida binding protein	1.060773
LDB3	10q22.3-q23.2	LIM domain binding 3	1.202968
SNX26	19q13.13	sorting nexin 26	1.323889
ITGB2	21q22.3	integrin, beta 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1; macrophage antigen 1 (mac-1) beta subunit)	1.486489
HP	16q22.1	haptoglobin	1.578568
RAFTLIN	3p25.1	raft-linking protein	1.697333
PPARD	6p21.2-p21.1	peroxisome proliferative activated receptor, delta	2.160981

Table 2. Genes Showing Melittin-induced Down-regulation in Human Mast Cells

Gene	Chromosome	Title	Global M
LCMR1	11q12	lung cancer metastasis-related protein 1	-1.52951
CREB3L4	1q22	cAMP responsive element binding protein 3-like 4	-1.36901
C8B	1p32	complement component 8, beta polypeptide	-1.27894
PKD2	4q21-q23	polycystic kidney disease 2 (autosomal dominant)	-1.20995
RGS13	1q31.1	regulator of G-protein signalling 13	-1.15733
SCA7	3p21.1-p12	spinocerebellar ataxia 7 (olivopontocerebellar atrophy with retinal degeneration)	-1.13143
BCL3	19q13.1-q13.2	B-cell CLL/lymphoma 3	-1.12572
CYP2E1	10q24.3-qter	cytochrome P450, family 2, subfamily E, polypeptide 1	-1.11742

different expressions in all chips, there were 7 up-regulated and 17 down-regulated genes in the gene expression profiles of MCD peptide treated group(Table 3, 4).

IV. Discussion

Bee venom (BV) has been used for the treatment of inflammatory diseases such as rheumatoid arthritis and relief of pain in Oriental medicine[11]. BV possesses anti-in flammatory effects, a property of nonsteroidal anti-inflammatory drugs (NSAIDs)[12]. The venom from the gland of the honeybee, Apis Mellifera, produces an aqueous se cretion which contains in significant quantity only two enzymes and four peptides[13]. The enzymes are phospholipase A2(PLA2) and a hyalurodinase,

Table 3. Genes Showing MCD Peptide-induced Up-regulation in Human Mast Cells

Gene	Chromosome	Title	Global M
MGC10067	5q33.3	hypothetical protein MGC10067	1.031109
GABRA5	15q11.2-q12	gamma-aminobutyric acid (GABA) A receptor, alpha 5	1.106516
SLC12A3	16q13	solute carrier family 12 (sodium/chloride transporters), member 3	1.145855
NRIP1	21q11.2	nuclear receptor interacting protein 1	1.153284
RoXaN	22q13.2	ubiquitous tetratricopeptide containing protein RoXaN	1.157154
RAMP1	2q36-q37.1	receptor(calcitonin) activity modifying protein 1	1.192644
FLJ10525	4p14	hypothetical protein FLJ10525	1.282544

Gene expression was profiled with TwinchipTM Human-8K cDNA microarray.
Cut-off level of two-fold change was used for the gene up-regulated by MCD peptide treated.

Table 4. Genes Showing MCD peptide-induced Down-regulation in Human Mast Cells

Gene	Chromosome	Title	Global M
TM4SF5	17p 13.3	transmembrane 4 superfamily member 5	-2.0715
PML	15q22	promyelocytic leukemia	-1.74422
B3GALT3	3q25	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 3	-1.73462
KIAA1404	20q13.13	KIAA1404 protein	-1.60414
PGM1	1p31	phosphoglucomutase 1	-1.58086
PRB2	12p13.2	proline-rich protein BstNI subfamily 2	-1.50308
LAIR1	19q13.4	leukocyte-associated Ig-like receptor 1	-1.49653
ITIH3	3p21.2-p21.1	inter-alpha (globulin) inhibitor H3	-1.45254
GRB14	2q22-q24	growth factor receptor-bound protein 14	-1.43733
MSMB	10q11.2	microseminoprotein, beta-	-1.20415
ECH1	19q13.1	enoyl Coenzyme A hydratase 1, peroxisomal	-1.1739
PROC	2q13-q14	protein C (inactivator of coagulation factors Va and VIIIa)	-1.16753
PEX19	1q22	peroxisomal biogenesis factor 19	-1.1603
GABRG3	15q11-q13	gamma-aminobutyric acid (GABA) A receptor, gamma 3	-1.12662
GATA6	18q11.1-q11.2	GATA binding protein 6	-1.10937
MGC48322	5q23.3	hypothetical protein MGC48332	-1.07831
ERCC3	2q21	excision repair cross-complementing rodent repair deficiency, complementation group 3 (xeroderma pigmentosum group B complementing)	-1.01952

Gene expression was profiled with TwinchipTM Human-8K cDNA microarray.
Cut-off level of two-fold change was used for the gene down-regulated by MCD peptide treated.

and the amino acid sequence of these proteins has been determined. Among this, Melittin, the major active ingredient of BV[14], has been reported to induce apoptosis[15-16] and possess anti-inflammatory and anti-tumor effects[17]. It represents 50-60% of dry weight of whole BV. Other peptides, each present at less than one-tenth the amount of melittin, are mast cell-degranulating (MCD) peptide, apamin, and secapin. MCD peptide also called peptide 401 contains 22 amino acids. It has a potent histamin-releasing activity and also acts an anti-inflammatory agent. Upon injection into brain, MCD peptide binds with high affinity to voltage-sensitive potassium channel[18].

Melittin, the major component of bee venom, is a 26-amino acid cationic peptide that disrupts membranes and exhibits a strong cytotoxic activity[19]. In aqueous solution melittin forms amphipathic-helices that interact with lipid membranes via a positively charged cluster (Lys-Arg-Lys -Arg) near the C terminus, inserting into the lipid bilayer and perturbing the structure. These activities, combined with its net positive charge, make melittin an interesting candidate for enhancing the delivery of DNA in transfection protocols. However, early attempts using unmodified melittin as a transfection agent showed only slightly enhanced transgene expression because of the fast partitioning of melittin into cell membranes, with concomitant high toxicity. Plank and colleagues[20] recently showed that a minimum number of seven positive charges per polycation is necessary to prevent disassembly of polyelectrolyte DNA complexes under physiological salt concentrations; hence, dissociation of DNA complexes with melittin, which has only five positive charges, may be anticipated[21].

Mast cell degranulating (MCD) peptide, a cationic 22-amino acid residue peptide with two disulfide bridges, causes mast cell degranulation and histamine release at low concentrations and has anti-inflammatory activity at higher concentrations. Because of these unique immunologic properties, MCD peptide may serve as a useful tool for studying secretory mechanisms of inflammatory cells such as mast cells, basophils, and leukocytes, leading to the design of compounds with therapeutic potential[22].

Historically, mast cells have been implicated in two contrasting types of immune responses. First, they can be activated by immunoglobulin IgE receptors to mediate immediate hypersensitivity reactions associated with allergic phenomena. Second, their acute activation by microbial products, as in bacterial peritonitis models, underscores their role in infection[23]. Mast cells themselves produce a series of effector molecules that mediate permeability, inflammation, chemotaxis, and tissue destruction. They are the only cells that contain preformed TNF-a in granules and they also display an ability to rapidly produce large amounts of both TNF-a and IL-1, cytokines that play a critical role in K/BxN arthritis, as well as in human rheumatoid arthritis. Mast cell granules also contain an abundance of proteases capable of activating matrix metalloproteinases and mMCP-6, a potent indirect neutrophil chemoattractant. These cells also produce large quantities of other inflammatory molecules including histamine, eicosanoids, fibroblast growth factor, and angiogenesis factors (VEGF), which may contribute further to the arthritic process. Mast cells reside in tissues and generally possess a centrally positioned, oval nucleus. The mast cells in the lung and skin of most normal individuals contain chymase, carboxypeptidase A, elastase, cathepsin G, and multiple tryptases in their granules[24].

The MTT assay is conventionally used for measuring cell proliferation, however, because the metabolic conversion of the MTT salt is mediated by active mitochondrial dehydrogenase in living cells, the test can also be used to assess cell viability. It relies upon the cellular reduction of tetrazolium salts to their intensely colored formazans. In this method, percent cell viability (optical density of experiment group/optical density of control group ×100) represented cytotoxic effects. In order to find out the concentration at which the cytotoxic effects of melittin or MCD peptide on the human mast cell lines becomes evident, analysis by MTT assay was performed. In melittin treated groups, concentrations of 10-2, 10-1, 1, 10 mg/ml groups were significant compared with control group. It was used the 1 mg/ml concentration treated group for study. In MCD peptide treated groups, concentrations of 10-2, 10-1, 1 mg/ml groups were significant compared with control group. It was used the 0.1 mg/ml concentration treated group for study.

DNA microarray technology is a new and promising tool that allows the detection of several hundred or even thousands DNA sequences simultaneously for high throughput comprehensive analysis of thousand of genes in parallel. Recent advances in sample processing and DNA microarray technologies provide new perspectives to assess microbial water quality[25]. Profiling of gene expression patterns with microarray technology is widely used in both basic and applied research. DNA microarrays have also shown great promise in clinical medicine and are paving the way toward effective pharmaceutical drug discovery and individualized drug regimens. With growing utilization of this high-throughput technology, new applications are making headlines on a regular basis[26].

Microarray analysis has been emerged as a tool to characterize the overall reaction of cells in culture or tissue to different stimuli e.g. stressful events by analysing bulk RNA present at a particular time point. It has supplemented or even replaced more traditional methods like cDNA-bank sequencing or conventional differential display. The commercial availability of several different precoated arrays and the ease of handling has supported the broad distribution of this new technique. The basic protocol involves the hybridization of complementary strands of labelled DNA or RNA from cells/tissue with representations of known genes spotted onto a solid support (nylon, glass). Labelling can be radioactive, by a hapten group (biotin, digoxigenin, aminoallyl) or by fluorescent (Cy3, Cy5 etc.) nucleotides. Detection is performed by autoradiography, chemiluminescence or fluorescence scanning. There are different setups of arrays available: either known genes/gene-groups (apoptosis, cytokines etc.) are spotted as PCR fragments, plasmids or synthetic oligonucleotides or representations of the known genome are directly synthesized as short sequence tags of 20-70 oligonucleotides on glass chips. The latter allow the identification of newly expressed genes whereas the former deal with known genes. Ideally, the intensity of the signal can be correlated with the relative expression of a known gene and allows the comparison with a standard. Problems arise from the quality of the sample material, the standardization of the protocols and the data management. Nevertheless, gene profiling by cDNA-arrays will definitely be integrated into routine screening programs[27]. The application of a DNA microarray to compare normal and pathological cells, tissues or organs may allow, along with classical positional cloning techniques, to speed up the

discovery of genes and gene pathways implicated in several diseases[28].

In this present study, it was performed an analysis on TwinchipTM Human-8K in order to acquire the gene expression profiles of melittin and MCD peptide on human mast cells. TwinchipTM Human-8K chip consists 7381 genes, and divide about 8 groups according to their functions. The major group is related to cell growth and maintenance, come under about 18%. Other groups are as follows: signal transduction 14%, cell cycle 4%, cell death 2%, response to stress 4%, immune response 5%, transcription 8%, apoptosis 2%, and others 43%.

In the gene expression profiles of melittin treated group, there were 7 up-regulated and 8 down-regulated genes. Up-regulated genes were ZPBP, LDB3, SNX26, ITGB2, HP, RAFTLIN, and PRARD and down-regulated genes were LCMR1, CREB3L4, C8B, PKD2, RGS13, SCA7, BCL3, and CYP2E1. Up-regulated genes are related to the follow substances; binding protein in cell, lymphocyte function-associated antigen, macrophage antigen, peroxosome proliferative activated receptor. Down-regulated genes are related to the follow substances; lung cancer metastasis-related protein, cAMP responsive element binding protein. complement component 8, polycystic kidney disease 2 (autosomal dominant), regulator of G-protein signalling 13, spinocerebellar ataxia (olivopontocerebellar atrophy with retinal degeneration), B-cell leukemia, polypeptide related drug metabolism and synthesis of cholesterol, steroids and other lipids.

In the gene expression profiles of MCD peptide treated group, there were 7 up-regulated and 17 down-regulated genes. Up-regulated genes were MGC10067, GABRA5, SLC12A3, NRIP1, RoXan, RAMP1, and

FLJ10525 and down-regulated genes were TM4SF5, PML, B3GALT3, KIAA1404, PGM1, PRB2, LAIR1, ITIH3, GRB14, MSMB, ECH1, PROC, PEX19, GABRG3, GATA6, MGC48322, and ERCC3. Up-regulated genes are related to the follow substances; hypothetical protein, gamma-aminobutyric acid A receptor, solute carrier family 12, nuclear receptor interacting protein, ubiquitous tetratricopeptide containing protein, receptor activity modifying protein, hypothetical protein. Down-regulated genes are related to the follow substances; transmembrane 4 superfamily member 5, promyelocytic leukemia, betaGLcNAc beta 1,3-galactosyltransferase, polypeptide 3, phosphoglucomutase, proline-rich protein, leukocyre-associated Ig-like receptor, knter-alpha globulin inhibitor, growth factor receptor-bound protein, microseminoprotein, enoyl Coenzyme A hydratase1, peroxisomal protein C, peroxismol biogenesis factor 19, gamma-aminobutyric acid A receptor, GATA binding protein, hypothetrical protein, excision repair cross-complementing rodent repair deficiency, and complementation group 3.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors involved in the regulation of lipid and glucose metabolism. Three distinct PPARs, termed alpha (PPARA), gamma (PPARG), and delta (PPARD), have been isolated. They are encoded by separate genes and characterized by distinct tissue and developmental distribution patterns. PPARD is, in contrast with the other two PPARs, ubiquitously expressed[29]. PPARA is involved in lipid metabolism and fatty acid oxidation whereas PPARG influences adipocyte differentiation and insulin action[30]. The function of PPARD is not yet fully understood, but it has been shown to play a role in cholesterol metabolism in animal models. Treatment with a PPARD agonist

increased plasma cholesterol concentrations in mice with a dysfunctional leptin receptor (db/db mice)[31]. A recent study showed that treatment with a potent selective PPARD agonist increased plasma cholesterol concentrations, decreased plasma triglyceride concentrations, increased HDL cholesterol, and decreased the fraction of small and dense LDLs in obese rhesus monkeys[32]. Furthermore, PPARD plays a central role in fatty acid-controlled differentiation of preadipocytes, where it promotes induction of PPARG and other genes involved in adipocyte differentiation[33]. Taken together, these data suggest that PPARD may play a role in the development of metabolic perturbations associated with dyslipidemia and predisposing to atherosclerosis.

Integrins constitute a large family of receptors that mediate adhesive interactions between cells or between a cell and the extracellular matrix. Early characterization of integrins focused on the physical link they provide between the extracellular matrix and the cytoskeleton[34]. However, it has become apparent that integrin adhesion receptors regulate many other fundamental biological processes as well. In particular, integrins can act as signaling receptors that transmit information about the extracellular environment to the interior of the cell, affecting many important aspects of cell behavior, such as survival, proliferation, motility, and differentiation[35]. Increasing evidence shows that integrin engagement can regulate transcription either through direct signaling to the nucleus or through biochemical elements shared with other signaling receptors, such as the Janus tyrosine kinase-signal transducers and activators of transcription or the MAPK1 pathways. Each integrin consists of a heterodimer of two transmembrane glycoproteins, an and a chain. Integrin LFA-1

(lymphocyte function-associated antigen-1), a heterodimer of the L and the 2 subunits, is essential in many stages of the immune response, such as transmigration processes or the adhesive and signaling events that occur in the immunological synapse[36].

The eighth component of human complement (C8) is a serum protein that consists of three chains (alpha, beta and gamma), encoded by three separate genes, viz., C8A, C8B, and C8G. In serum, the beta-subunit is non-covalently bound to the disulfide-linked alpha-gamma subunit. C8 beta-cDNA-positive clones were partially sequenced to characterize the 12 exons of the gene with sizes from 69 to 347 bp. All intron-exon junctions followed the GT-AG rule. By using polymerase chain reaction (PCR) primers located in the adjacent intron sequences, all 12 exons of the C8B gene could be amplified from genomic DNA. All fragments showed the expected sizes. The sizes of eight introns could be determined by using primer pairs that amplified two exons and the enclosed intron, and by restriction mapping. These analyses and the insert sizes of the genomic lambda clones indicate that the C8B gene has a total size of approximately 40 kb. The polymorphic TaqI site of the C8B gene localized in intron 11 could be demonstrated by direct restriction fragment analysis of a PCR fragment containing exons 11 and 12, and the enclosed intron 11. Homology comparison of the C8B gene with C8A and C9 on the basis of the exon structure confirmed the ancestral relationship known from the protein level[37].

g-Aminobutyric acid (GABA), the principal inhibitory neurotransmitter in mammalian brain, exerts its effects through GABAA receptors that are ligand-gated ion channels. Functional GABAA receptors appear to be composed of five homologous, variable subunits arranged to

form a central channel that conducts the chloride ions through the cell membrane. Each subunit consists of a long, variable extracellular region, four highly conserved transmembrane regions, and a variable intercellular region between the third and the fourth transmembrane regions[38].

The g-aminobutyric acid-type A receptor a5 subunit gene (GABRA5) is widely expressed in brain and localized to the imprinted human chromosome 15q11-q13. A combination of cDNA library screening and 5'RACE analysis led to identification of three distinct mRNA isoforms of GABRA5 in human adult and fetal brain tissues, each of which differs only in the noncoding 5'UTR sequence[39].

These genes probably relate to the effects of melittin and MCD peptide, and further studies are needed to find out the gene informations.

V. Conclusion

To investigate gene expression profiling of melittin and MCD peptide, in human mast cell strain, it was used cDNA microarray analysis and then observed the results as follows.

1. In the gene expression profiles of melittin treated group, there were 7 up-regulated and 8 down-regulated genes. Up-regulated genes were ZPBP, LDB3, SNX26, ITGB2, HP, RAFTLIN, and PRARD and down-regulated genes were LCMR1, CREB3L4, C8B, PKD2, RGS13, SCA7, BCL3, and CYP2E1.
2. In the gene expression profiles of MCD peptide treated group, there were 7 up-regulated and 17 down-regulated genes. Up-regulated genes were MGC10067,

GABRA5, SLC12A3, NRIP1, RoXan, RAMP1, and FLJ10525 and down-regulated genes were TM4SF5, PML, B3GALT3, KIAA1404, PGM1, PRB2, LAIR1, ITIH3, GRB14, MSMB, ECH1, PROC, PEX19, GABRG3, GATA6, MGC48322, and ERCC3.

This study presented the gene expression of bee venom major constituents, it would be the basis of further more gene study to investigate the functions of melittin and MCD peptide.

VI. References

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