

Classification of the Efficacy of Herbal Medicine Alterations in Neuronal Hypoxia Models through Analysis of Gene Expression

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Objectives: cDNA microarray is an effective method to snapshot gene expression. Functional clustering of gene expressions can identify herbal medicine mechanisms. Much microarray data is available for various herbal medicines. This study compares regulated genes with herbal medicines to evaluate the nature of the drugs.

Methods: Published microarray data were collected. Total RNAs were prepared from dissociated hippocampal dissociate cultures which were given hypoxic shock in the presence of each herbal medicine. Up- or downregulated genes higher than Global M value 0.5 were selected, clustered in functional groups, and compared with various herbal treatments.

Results: 1. Akt2 was upregulated by *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI and *Coptis chinensis* FRANCH, and they belong to Araceae herb. 2. NF- κ b1, Cd5, Gn γ 7 and Sgnt1 were upregulated by *Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH and *Rheum coreanum* NAKAI. 3. *Woohwangcheongsim-won*, *Sohaphyang-won* and *Scutellaria baicalensis* GEORGI downregulated Scp2 and upregulated Tsc2. *Woohwangcheongsim-won* and *Sohaphyang-won* upregulated Hba1 and downregulated Myf6. 4. *Sohaphyang-won* and *Scutellaria baicalensis* GEORGI downregulated Slc12a1. 5. *Woohwangcheongsim-won* and *Arisaema amurense* var. *serratum* NAKAI upregulated Rar α , *Woohwangcheongsim-won* and *Coptis chinensis* FRANCH downregulated Rab5a and Pdgfr α , and *Woohwangcheongsim-won* and *Rheum coreanum* NAKAI upregulated Plc γ 1 and downregulated Pla2g1b and Slc10a1.

Conclusions: By clustering microarray, genes are commonly identified to be either up- or downregulated. These results will provide new information to understand the efficacy of herbal medicines and to classify them at the molecular level.

Key Words : Hypoxia, apoptosis, gene expression, microarray

Introduction

Apoptosis, due to reactive oxygen species (ROS), serves an important role in degenerative disorders of the nervous system. Because apoptosis results in numerous semi-permanent side effects even after treatment, there has been a lot of research on a

treatment method that can effectively decrease apoptosis^{1,2)}.

Research on discovering biological, pathological mechanisms through genetic regulation has been increasing since the completion of the genetic map of several species, including that of human beings^{3, 4)}. Microarray, a technique to find the function and

• Received : 16 November 2014

• Revised : 17 December 2014

• Accepted : 17 December 2014

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structure of genes, makes it possible to determine the general expression of an entire gene within a cell. It is a major tool in function genomics research^{5,6}.

Previous experimental research reports that some herbal medicines or extracts delay apoptosis due to hypoxia, and the medicine's gene expression has been studied through the microarray. By analyzing the function of genes where alteration in expression occurred and the neuron's genetic expression alteration by each medicine and treatment, it is possible to infer the medicine's mechanism⁷⁻¹⁴. However, although each herbal medicine may have functioned by different mechanisms, there has been no report about the instance of mutual comparison and interpretation by merging Korean medicine theory.

The author analyzed experimental studies that observed expressed genes from herbal medicines or extracts in neurons where apoptosis has occurred under hypoxia and interpreted the influence on gene function related to the medicine's neuron protection and anti-oxidation from the point of view of Korean medicine.

Research Target and Method

1. Research Target

Clustering is an analyzing method that classifies the genes that have been identified with expression alteration through microarray by similar groups. Hierarchical clustering is a method that groups genes with similar characteristics and analyzes them. It is useful in understanding a herbal medicine and treatment's properties based on the expressed genes¹⁶.

This study limited its target to experimental research that used clustering to investigate altered expression of genes through microarray after inducing apoptosis in neurons under hypoxia and treating with herbal medicines or extracts.

As a result, with similar analyzing methods, we

detected results from observing gene alteration. We compared and analyzed based on the research findings that use *Acorus gramineus* SOLAND⁷, *Arisaema amurense* var. *serratum* NAKAI⁸, *Pinellia ternata* BREIT.⁹, *Scutellaria baicalensis* GEORGI¹⁰, *Coptis chinensis* FRANCH¹¹, *Rheum coreanum* NAKAI¹², *Woohwangcheongsim-won*¹³, and *Sohaphyang-won*¹⁴, that are frequently used in cerebrovascular disease.

2. Research Method

Among the genes expressed through microarray, we compared expressed genes with global M figure higher than +0.5 and lower than -0.5. We compared *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI, and *Pinellia ternata* BREIT., which belong to the araceae herb and has function of dispel *Dam-eum* (phlegm-retained fluid); and compared *Pinellia ternata* BREIT., *Coptis chinensis* FRANCH, and *Rheum coreanum* NAKAI that are expressed in similar class; and observed whether there is an overlap of expressed genes between different medicines. Then, we compared the gene expression of *Woohwangcheongsim-won* and *Sohaphyang-won* that are used for acute stroke and observed an overlap of expressed genes with other medicines.

Results

1. Comparison of microarray methods

Table 1 shows the microarray method and the mRNA separation process from neurons induced of apoptosis due to ischemic damage from each study.

2. Clustering of herbal medicine's genes

Table 2 shows the classification of each herbal medicine based on reported research findings. We excluded findings of global M figures within ± 0.5 because there is a difference in the global M figure which is the standard for each study.

Table 1. Comparison of Experimental Procedures for Microarrays of Each Herb Extract or Composites.

	Concentration	Added DIV	Inducing hypoxia	Microarray platform	Company	Reference
<i>Acorus gramineus</i> SOLAND	10µg/ml	DIV 12	DIV 14	TwinChip™ Rat-5K	Digital Genomics	Park et al. (2007) ⁷
<i>Arisaema amurense</i> var. <i>serratum</i> NAKAI	10µg/ml	DIV 12	DIV 14	TwinChip™ Rat-5K	Digital Genomics	Koh et al. (2003) ⁸
<i>Pinellia ternata</i> BREIT.	2.5µg/ml	DIV 12	DIV 14	TwinChip™ Rat-5K	Digital Genomics	Kwon et al. (2005) ⁹
<i>Scutellaria baicalensis</i> GEORGI	20µg/ml	DIV 12	DIV 14	TwinChip™ Rat-5K	Digital Genomics	Kim et al. (2004) ¹⁰
<i>Coptis chinensis</i> FRANCH	2.5µg/ml	DIV 12	DIV 14	TwinChip™ Rat-5K	Digital Genomics	Hwang et al. (2005) ¹¹
<i>Rheum coreanum</i> NAKAI	2.5µg/ml	DIV 10	DIV 13	Rat 44K 4-Plex Gene Expression platform(Agilent)	Digital Genomics	Lee et al. (2009) ¹²
<i>Woohwangcheongsim-won</i>	20µg/ml	DIV 12	DIV 14	TwinChip™ Rat-5K	Digital Genomics	Park et al. (2004) ¹³
<i>Sohaphyang-won</i>	20µg/ml	DIV 12	DIV 14	TwinChip™ Rat-5K	Digital Genomics	Paik et al. (2004) ¹⁴

All experiments were performed using E18 rat embryonic hippocampal neurons in culture grown in the neurobasal medium supplemented with B27.

All components were diluted or extracted with water and added directly to the medium.

* In all cases hypoxic shock was given for 3 hrs in 2% O2/5% CO2 and total mRNAs were extracted 24 hrs after shock.

Table 2. The Number of Genes Up- or Downregulated by Each Treatment

		<i>Acorus gramineus</i> SOLAND	<i>Arisaema amurense</i> var. <i>serratum</i> NAKAI	<i>Pinellia ternata</i> BREIT.	<i>Scutellaria baicalensis</i> GEORGI	<i>Coptis chinensis</i> FRANCH	<i>Rheum coreanum</i> NAKAI	<i>Woohwangche-ongsim-won</i>	<i>Sohaphyang-won</i>
Apoptosis	upregulated		1			12	6		
	downregulated	1		2		5	6		
Growth & maintenance	upregulated	6	17		2			6	3
	downregulated	8	4		3			18	3
Cell cycle	upregulated	1	2	1		15	5		
	downregulated		1	1	1	15	8	1	
Response to stress	upregulated	1	5	1		27	17	5	
	downregulated			1		16	11	2	
Signal transduction	upregulated		12	3	1	56	22	5	1
	downregulated	2	2	2	1	41	16	6	
Transcription	upregulated	1	4	1	1	20	11	2	
	downregulated		2	3		15	17	5	1
Physiological process	upregulated			8		185	24		
	downregulated			16		129	12		
Immune response	upregulated		3	1		19	11		1
	downregulated	1				10	6	3	

3. Analysis of gene alternation between medicines

We investigated genes that demonstrate similar alteration between medicines.

1) Comparison of global M figure of *Acorus gramineus* SOLAND and *Arisaema amurense* var. *serratum* NAKAI

Thymoma viral proto-oncogene 2 (Akt2) altered in the same direction in the research on *Acorus gramineus* SOLAND and *Arisaema amurense* var. *serratum* NAKAI, which belong to the araceae herb. Table 3 shows the result of the analysis.

2) Comparison of global M figure of *Acorus gramineus* SOLAND and *Coptis chinensis* FRANCH

Akt2 altered in the same direction in the research

on *Acorus gramineus* SOLAND and *Coptis chinensis* FRANCH. Table 4 shows the result of the analysis.

3) Comparison of global M figure of *Coptis chinensis* FRANCH and *Pinellia ternata* BREIT.

CD3 molecule, delta (Cd3 δ) altered in the same direction in the research on *Coptis chinensis* FRANCH and *Pinellia ternata* BREIT. Table 5 shows the result of the analysis.

4) Comparison of global M figure of *Coptis chinensis* FRANCH and *Arisaema amurense* var. *serratum* NAKAI

A total of 25 types of genes were altered in the identical direction. Table 6 shows the result of the analysis. 2 types of genes related to apoptosis, 13 types of genes related to growth & maintenance, 2

Table 3. An Upregulated Gene by Both *Acorus gramineus* SOLAND and *Arisaema amurense* var. *serratum* NAKAI

		<i>Acorus gramineus</i> SOLAND	<i>Arisaema amurense</i> var. <i>serratum</i> NAKAI
Growth & maintenance	upregulated		1
	downregulated		-
Cell cycle	upregulated		1
	downregulated		-

Table 4. An Upregulated Gene by Both *Acorus gramineus* SOLAND and *Coptis chinensis* FRANCH

		<i>Acorus gramineus</i> SOLAND	<i>Coptis chinensis</i> FRANCH
Growth & maintenance	upregulated		1
	downregulated		-
Cell cycle	upregulated		1
	downregulated		-

Table 5. An Upregulated Gene by Both *Coptis chinensis* FRANCH and *Pinellia ternata* BREIT

		<i>Coptis chinensis</i> FRANCH	<i>Pinellia ternata</i> BREIT.
Signal transduction	upregulated		1
	downregulated		-
Physiological process	upregulated		1
	downregulated		-
Immune response	upregulated		1
	downregulated		-

types of genes related to cell cycle, 4 types of genes related to response to stress, 11 types of genes related to signal transduction, 3 types of genes related to transcription, 18 types of genes related to physiological process, and 3 types of genes related to immune response.

5) Comparison of global M figure of *Coptis chinensis* FRANCH and *Rheum coreanum* NAKAI

A total of 29 types of genes were altered in the identical direction. Table 7 shows the result of the analysis. There were 2 types of genes related to apoptosis, 3 types of genes related to cell cycle, 10 types of genes related to response to stress, 11 types of genes related to signal transduction, 5 types of genes related to transcription, 17 types of genes related to physiological process, and 7 types of genes related to immune response.

6) Comparison of global M figure of *Arisaema amurense* var. *serratum* NAKAI and *Rheum coreanum* NAKAI

A total of 5 types of genes were altered in the identical direction. Table 8 shows the result of the analysis. There were 1 type of gene related to apoptosis, 1 type of gene related to growth & maintenance, 2 types of genes related to response to stress, 3 types of genes related to signal transduction, 2 types of genes related to transcription, 2 types of genes related to physiological process, and 2 types of genes related to immune response.

7) Comparison of global M figure of *Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH and *Rheum coreanum* NAKAI

A total of 4 types of genes were altered in the identical direction in *Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH, and *Rheum coreanum* NAKAI. Table 9 shows the result of the analysis.

4. Comparison between *Woohwangcheongsim-won* and *Sohaphyang-won* and each

Table 6. Genes Up- or Downregulated by Both *Coptis chinensis* FRANCH and *Arisaema amurense* var. *serratum* NAKAI

		<i>Coptis chinensis</i> FRANCH	<i>Arisaema amurense</i> var. <i>serratum</i> NAKAI
Apoptosis	upregulated		2
	downregulated		-
Growth & maintenance	upregulated		13
	downregulated		-
Cell cycle	upregulated		2
	downregulated		-
Response to stress	upregulated		4
	downregulated		-
Signal transduction	upregulated		10
	downregulated		1
Transcription	upregulated		3
	downregulated		-
Physiological process	upregulated		18
	downregulated		-
Immune response	upregulated		3
	downregulated		-

medicine

We compared the genes that are identical from the research findings on *Woohwangcheongsim-won* and *Sohaphyang-won* which are used for acute stroke

and compared the genes that were up- and down-regulated in the same direction from the research findings with other medicines.

Table 7. Genes Up- or Downregulated by Both *Coptis chinensis* FRANCH and *Rheum coreanum* NAKAI

		<i>Coptis chinensis</i> FRANCH	<i>Rheum coreanum</i> NAKAI
Apoptosis	upregulated		2
	downregulated		-
Cell cycle	upregulated		3
	downregulated		-
Response to stress	upregulated		10
	downregulated		-
Signal transduction	upregulated		10
	downregulated		1
Transcription	upregulated		4
	downregulated		1
Physiological process	upregulated		17
	downregulated		-
Immune response	upregulated		6
	downregulated		1

Table 8. Genes Upregulated by *Arisaema amurense* var. *serratum* NAKAI, and *Rheum coreanum* NAKAI

		<i>Coptis chinensis</i> FRANCH	<i>Rheum coreanum</i> NAKAI
Apoptosis	upregulated		1
	downregulated		-
Growth & maintenance	upregulated		1
	downregulated		-
Cell cycle	upregulated		
	downregulated		
Response to stress	upregulated		1
	downregulated		-
Signal transduction	upregulated		3
	downregulated		-
Transcription	upregulated		2
	downregulated		-
Physiological process	upregulated		2
	downregulated		-
Immune response	upregulated		2
	downregulated		-

1) A total of 4 types of genes were identical. Table 10 shows the result of the analysis. There were 3 types of genes related to growth & maintenance, 1 type of gene related to signal transduction, and 1 type of gene related to transcription.

2) Comparison of global M figure of *Woohwangcheongsim-won* and *Scutellaria baicalensis* ^{G_{GEORGI}}

Tuberous sclerosis 2 (Tsc2) and sterol carrier protein 2 (Scp2) were altered in the identical direction in the research on *Woohwangcheongsim-won* and *Scutellaria baicalensis* ^{G_{GEORGI}}. Table 11

shows the analysis result.

3) Comparison of global M figure of *Sohaphyang-won* and *Scutellaria baicalensis* ^{G_{GEORGI}}

A total of 3 types of genes were identical. Table 12 shows the result of the analysis. There were 3 types of genes related to growth & maintenance and 1 type of gene related to signal transduction. Fig. 4 shows the genes that are upregulated in the three studies on *Woohwangcheongsim-won*, *Sohaphyang-won* and *Scutellaria baicalensis* ^{G_{GEORGI}}.

4) Comparison of global M figure of *Woohwangcheongsim-won* and *Arisaema amurense*

Table 9. Genes Upregulated by *Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH and *Rheum coreanum* NAKAI

		<i>Arisaema amurense</i> var. <i>serratum</i> NAKAI	<i>Coptis chinensis</i> FRANCH	<i>Rheum coreanum</i> NAKAI
Apoptosis	upregulated		1	
	downregulated		-	
Growth & maintenance	upregulated		1	
	downregulated		-	
Response to stress	upregulated		2	
	downregulated		-	
Signal transduction	upregulated		3	
	downregulated		-	
Transcription	upregulated		1	
	downregulated		-	
Physiological process	upregulated		1	
	downregulated		-	
Immune response	upregulated		2	
	downregulated		-	

Table 10. Genes Up- or Downregulated by Both *Woohwangcheongsim-won* and *Sohaphyang-won*

		<i>Woohwangcheongsim-won</i>	<i>Sohaphyang-won</i>
Growth & maintenance	upregulated		2
	downregulated		1
Signal transduction	upregulated		1
	downregulated		-
Transcription	upregulated		-
	downregulated		1

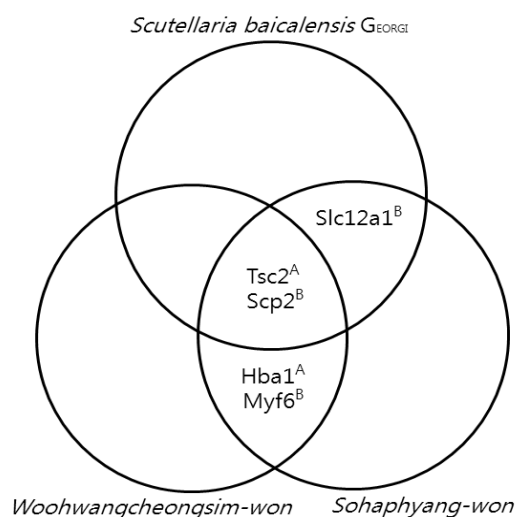


Fig. 1. Gene distribution expressed by *Scutellaria baicalensis* GEORGI, *Woohwangcheongsim-won* and *Sohaphyang-won*. A. Upregulated genes. B. downregulated genes.

var. *serratum* NAKAI

Retinoic acid receptor, alpha ($Rar\alpha$) was altered in the identical direction in the research on *Woohwangcheongsim-won* and *Arisaema amurense* var. *serratum* NAKAI. Table 13 shows the analysis result.

5) Comparison of global M figure of *Woohwangcheongsim-won* and *Coptis chinensis* FRANCH

Ras-related protein 4a (Rab4a) and platelet derived growth factor receptor, alpha polypeptide ($Pdgfr\alpha$) were altered in the identical direction in the research on *Woohwangcheongsim-won* and *Coptis chinensis* FRANCH. Table 14 shows the analysis result.

6) Comparison of global M figure of *Woohwangcheongsim-won* and *Rheum coreanum* NAKAI

Table 11. Genes Up- or Downregulated by Both *Woohwangcheongsim-won* and *Scutellaria baicalensis* GEORGI

		<i>Woohwangcheongsim-won</i>	<i>Scutellaria baicalensis</i> GEORGI
Growth & maintenance	upregulated		1
	downregulated		1
Signal transduction	upregulated		1
	downregulated		-

Table 12. Genes Up- or Downregulated by Both *Sohaphyang-won* and *Scutellaria baicalensis* GEORGI

		<i>Sohaphyang-won</i>	<i>Scutellaria baicalensis</i> GEORGI
Growth & maintenance	upregulated		1
	downregulated		2
Signal transduction	upregulated		1
	downregulated		-

Table 13. An upregulated Gene by Both *Woohwangcheongsim-won* and *Arisaema amurense* var. *serratum* NAKAI

		<i>Woohwangcheongsim-won</i>	<i>Arisaema amurense</i> var. <i>serratum</i> NAKAI
Apoptosis	upregulated		1
	downregulated		-
Transcription	upregulated		1
	downregulated		-

A total of 3 types of genes were identical. Table 15 shows the result of the analysis. There were 2 types of genes related to growth & maintenance, 1 type of gene related to response to stress and 1 type of gene related to signal transduction.

Discussion

There has been a lot of research on preventing apoptosis due to damage because regeneration is difficult once the neuron has been damaged. When a neuron is damaged due to ischemia/hypoxia, along with necrosis in the center, apoptosis in the penumbra occurs^{17,18}. Although apoptosis in the center is unpreventable because it occurs quickly, the penumbra goes through a delayed neuronal death, allowing time to minimize apoptosis¹⁹. Delayed neuronal death occurs through diverse mechanisms, but the main process is the production of ROS due to excitotoxicity. In other words, under hypoxia, there is an over-release of glutamate which over-activates the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor and N-methyl-D-aspartate (NMDA) receptor that pass Ca^{2+} , thereby

activating the apoptotic protein^{20,21}. When the calcium ion is overly entered into the mitochondria in the neuron, ROS is produced. Because of the overly produced ROS, the permeability of the mitochondria is altered, and the mitochondrial membrane potential (MMP) is lost. As a result, energy (ATP) production is hindered, and apoptosis occurs^{2,21,22}. Meanwhile, the neuron, under hypoxia, operates a self-defense system¹⁷ - it boosts angiogenesis, activates apoptosis defense protein and cell survival protein, etc. - in order to protect the neuron²³.

Recently, there has been a lot of research on the protective effects on the neuron of herbal medicines or extracts. Most look for pathological mechanisms and gene expression processes under the assumption that a particular protein expression alteration induces a pathological mechanism. However, this method does not consider the mutual interaction between proteins but focuses on the fragmentary role, which makes it difficult to know the overall connection because limited mechanisms can be understood even with the result.

cDNA microarray is a method that looks at large scale gene expression alteration simultaneously by

Table 14. Genes downregulated by Both *Woohwangcheongsim-won* and *Coptis chinensis* FRANCH

	<i>Woohwangcheongsim-won</i>	<i>Coptis chinensis</i> FRANCH
Growth & maintenance	upregulated	-
	downregulated	1
Signal transduction	upregulated	
	downregulated	2

Table 15. Genes Up- or Downregulated by Both *Woohwangcheongsim-won* and *Rheum coreanum* NAKAI

	<i>Woohwangcheongsim-won</i>	<i>Rheum coreanum</i> NAKAI
Growth & maintenance	upregulated	-
	downregulated	2
Response to stress	upregulated	
	downregulated	1
Signal transduction	upregulated	1
	downregulated	-

using a DNA chip to integrate the gene in high density. It is a high-tech gene analysis method that allows understanding of diverse alterations in a neuron during pathological process at the same time and observation of the overall alteration expression⁶. Also, because cDNA microarray estimates the amount of expression of the gene by the quantitative alteration strength between the experimental group and the control group, it finds the expression direction and the strength of gene expression in a short period of time. Therefore, recently it has been used in diverse bio-science research²⁴. Meanwhile, there are many methods to analyze the expression types of genes found from the cDNA microarray results, but to discover the mutual relationship of genes, the clustering method which groups by class according to their functions is often used. In other words, the clustering method has an advantage of knowing the medicine's point of action from a wider point of view because it observes the overall directionality rather than the individual gene expression²⁵.

There recently has been a lot of research that tries to find the characteristics of herbal medicines or extracts through cDNA microarray. This study compared and analyzed research reports limited to those that observed gene expression alteration through the clustering method in the neuronal hypoxia model among research on the gene expression of herbal medicines using the cDNA microarray method. Target herbal medicines were *Acorus gramineus* SOLAND⁷, *Arisaema amurense* var. *serratum* NAKAI⁸, *Pinellia ternata* BREIT.⁹, *Scutellaria baicalensis* GEORGI¹⁰, *Coptis chinensis* FRANCH¹¹, *Rheum coreanum* NAKAI¹², *Woohwangcheongsim-won*¹³, and *Sohaphyang-won*¹⁴. We interpreted their significance by discovering the genes that were upregulated in common by these medicines through the clustering analysis method of cDNA microarray.

From previous research, it was difficult to find a common ROS-producing cell for *Acorus gramineus*

SOLAND, *Arisaema amurense* var. *serratum* NAKAI, *Pinellia ternata* BREIT., *Scutellaria baicalensis* GEORGI, *Coptis chinensis* FRANCH, *Woohwangcheongsim-won*, and *Sohaphyang-won* because of excellent ROS removal ability, and they were helpful in maintaining MMP. In the case of *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI, *Scutellaria baicalensis* GEORGI, *Woohwangcheongsim-won*, and *Sohaphyang-won*, genes that remove ROS were expressed a lot. For example, in *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI, *Rar α* was upregulated, which is a gene that inhibits apoptosis^{7,8}. In research on *Woohwangcheongsim-won*, *Sohaphyang-won*, *Scutellaria baicalensis* GEORGI, and *Arisaema amurense* var. *serratum* NAKAI, catalase, which is an antioxidant enzyme, was upregulated^{8,10,13,14}. On the other hand, in *Pinellia ternata* BREIT., *Coptis chinensis* FRANCH, and *Rheum coreanum* NAKAI, genes that remove ROS were regulated relatively less than with *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI, *Scutellaria baicalensis* GEORGI, *Woohwangcheongsim-won*, and *Sohaphyang-won*; protein tyrosin phosphatase, non-receptor type 11 (Ptpn11) and Cd3 δ which are cell-supporting genes in their roots, and C-reactive protein (Crp) and platelet-activating factor receptor (Ptafr) which are genes related to the immune system, were upregulated a lot^{9,11,12}. In research on *Pinellia ternata* BREIT., tubulin, beta 5 (Tub β5), transforming growth factor alpha (Tgf α), Ptpn11, neuroblastoma ras oncogene (Nras), and platelet-derived growth factor alpha polypeptide (Pdgf α) which are related to cell growth and differentiation were upregulated⁹. In research on *Coptis chinensis* FRANCH, *Rheum coreanum* NAKAI, Defb3 which is a response to stress gene was upregulated and nuclear factor-kappaB (Nf-κb) which is related to neuron survival was upregulated^{11,12}.

In the study on *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI, and *Pinellia*

ternata BREIT., which are all medicines that belong to the araceae herb, through the cDNA microarray analysis research of *Acorus gramineus* SOLAND and *Arisaema amurense* var. *serratum* NAKAI, we observed that Akt2, which is known to be the most important gene in cell survival, was commonly upregulated. There were no observed genes that were up- or downregulated commonly when *Acorus gramineus* SOLAND, *Pinellia ternata* BREIT., *Pinellia ternata* BREIT., and *Arisaema amurense* var. *serratum* NAKAI were combined. Also, in *Acorus gramineus* SOLAND and *Coptis chinensis* FRANCH, only Akt2 was commonly upregulated. In sum, *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI, and *Coptis chinensis* FRANCH shared an upregulation of Akt2 [Global M = 0.9 (*Acorus gramineus* SOLAND), 0.6 (*Coptis chinensis* FRANCH), 0.7 (*Arisaema amurense* var. *serratum* NAKAI)].

Akt2 is an important gene in cell survival; it inhibits apoptosis in case of myogenic differentiation of serum removal state, and is known to inhibit apoptosis in divided cells²⁶). Therefore, from the fact that Akt2 was commonly upregulated in *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI, and *Coptis chinensis* FRANCH, we can infer that these medicines are related to effects of inhibiting apoptosis. *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI, and *Coptis chinensis* FRANCH all have controlled humidity function²⁷), and although it is difficult to conclude the function mechanism by Akt2 alone, it is a gene that should be considered for future research.

In *Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH, and *Rheum coreanum* NAKAI, four types of genes including Nf- κ b1 [Global M = 0.6 (*Arisaema amurense* var. *serratum* NAKAI), 2.0 (*Coptis chinensis* FRANCH), 1.2 (*Rheum coreanum* NAKAI)], Cd5 [Global M = 1.0 (*Arisaema amurense* var. *serratum* NAKAI), 1.7 (*Coptis chinensis* FRANCH), 0.5 (*Rheum coreanum* NAKAI)], Gng7 [Global M = 0.8 (*Arisaema amurense* var. *serratum* NAKAI), 1.6

(*Coptis chinensis* FRANCH), 0.6 (*Rheum coreanum* NAKAI)], and Sgse1 [Global M = 0.7 (*Arisaema amurense* var. *serratum* NAKAI), 0.7 (*Coptis chinensis* FRANCH), 0.7 (*Rheum coreanum* NAKAI)] were regulated in common. For Nf- κ b, p50 and p65 that are subunits are activated due to stimulus of glutamic acid in the neuron which accelerates apoptosis or p50, p65, c-Rel subunits are activated due to stimulus of interleukin (IL)-1 β which protects from apoptosis²⁸). If the density of inhibitor- κ B α (I κ B α) is decreased, IL-1 β stimulates Nf- κ b and protects the neuron²⁹). Caspase 3, caspase 21, Bcl2-associated X protein (Bax), which are apoptosis boosting genes, or IL-1 β , which is an apoptosis inhibiting gene, were not expressed in research of *Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH, and *Rheum coreanum* NAKAI. Caspase 2 (Casp2, Global M = -0.6) which is an apoptosis related gene was expressed in research of *Rheum coreanum* NAKAI. Unlike the caspase family, which is the general apoptosis boosting gene, because casp2 exhibits diverse functions such as acceleration or inhibition of apoptosis³⁰), even with the result of the upregulation of Nf- κ b, it is difficult to infer if the upregulation of Nf- κ b boosts or inhibits apoptosis. Guanine nucleotide binding protein, gamma 7 (Gn γ 7) which is a gene that produces guanine nucleotide binding protein (G protein) is known to exhibit downregulation in esophageal cancer or pancreatic cancer³¹). In this study, Gn γ 7 was commonly upregulated in *Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH, and *Rheum coreanum* NAKAI. These medicines do not share an identical function from the point of view of Korean Medicine. It is difficult to find a consistent function because we can only infer similar functions such as controlled humidity function (*Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH), dispelled *Dam-eum* (phlegm-retained fluid) (*Arisaema amurense* var. *serratum* NAKAI, *Rheum coreanum* NAKAI), and

dispelled *Dam-eum* (phlegm-retained fluid) (*Rheum coreanum* NAKAI). However, it is worth considering for future research as multiple genes were commonly expressed.²⁷⁾

For *Coptis chinensis* FRANCH, 25 types and 29 types of commonly expressed genes were each observed by the mutual comparison between *Coptis chinensis* FRANCH, *Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH, and *Rheum coreanum* NAKAI. From the result of *Coptis chinensis* FRANCH and *Pinellia ternata* BREIT., only Cd3 δ was expressed commonly and only *Woohwangcheongsim-won* and *Rab-4a*, *Pdgfr α* genes were expressed commonly. In reference to *Coptis chinensis* FRANCH, there are combined studies with *Scutellaria baicalensis* GEORGI, because it is expected to function similarly due to its clear heat effect. There was no gene that was expressed commonly between *Scutellaria baicalensis* GEORGI and *Coptis chinensis* FRANCH in this study. This result lends the possibility that *Coptis chinensis* FRANCH and *Scutellaria baicalensis* GEORGI may function in a different mechanism rather than the clear heat function in the same mechanism. Future research on this aspect is necessary.

From the observation results of common gene expression of *Sohaphyang-won* and *Scutellaria baicalensis* GEORGI, solute carrier family 12, member 1 (Slc12a1) [Global M = -0.5 (*Sohaphyang-won*), -1.1 (*Scutellaria baicalensis* GEORGI)], *Scp2* were expressed commonly. Also, from the observation result of *Woohwangcheongsim-won* and *Scutellaria baicalensis* GEORGI's gene expression, *Tsc2* and *Scp2* were found to be expressed commonly. In all *Scutellaria baicalensis* GEORGI, *Woohwangcheongsim-won* and *Sohaphyang-won*, *Tsc2* [Global M = 0.7 (*Woohwangcheongsim-won*), 0.5 (*Sohaphyang-won*), 0.6 (*Scutellaria baicalensis* GEORGI)] and *Scp2* [Global M = -1.3 (*Woohwangcheongsim-won*), -0.8 (*Sohaphyang-won*), -0.7 (*Scutellaria baicalensis* GEORGI)]'s expression alteration was observed.

Tsc2 is a tumor suppressor gene, and it stimulates cell growth when phosphorylated by Akt³²⁾. When there is low energy, AMP-activated protein kinase (AMPK) phosphorylates *Tsc2* and protects the cell from apoptosis³³⁾. In this study, in all *Woohwangcheongsim-won*, *Sohaphyang-won* and *Scutellaria baicalensis* GEORGI, *Tsc2* was upregulated, and this is identical to the apoptosis inhibiting function. Meanwhile, *Scp2* is a DNA combined protein that supports the chromosome structure in the former part of meiosis. It is involved in the transport and metabolism within the cholesterol cell and plays an important role in the production of macrophage foam cell which causes atherosclerosis³⁴⁾. Therefore, the downregulation of *Scp2* in *Woohwangcheongsim-won*, *Sohaphyang-won*, and *Scutellaria baicalensis* GEORGI research could be interpreted as a reduction of the lipid moving into the neuron³⁵⁾. Through this study, genes that are commonly expressed were identical in *Scutellaria baicalensis* GEORGI which is an ingredient medicine of *Woohwangcheongsim-won* and *Woohwangcheongsim-won* and *Sohaphyang-won*. Future research should study the gene that is expressed in the identical direction as the function research of the ingredient medicine of *Woohwangcheongsim-won* or *Sohaphyang-won*.

4 types of genes - hemoglobin, alpha 1 (Hb α 1) [Global M = 0.9 (*Woohwangcheongsim-won*), 1.7 (*Sohaphyang-won*)], *Tsc2*, *Scp2*, and myogenic factor 6 (Myf6) [Global M = -0.6 (*Woohwangcheongsim-won*), -0.5 (*Sohaphyang-won*)] - were found to be commonly expressed in the research on *Woohwangcheongsim-won* and *Sohaphyang-won*. Hb α 1, which is a type of hemoglobin that has oxygen transport ability, was upregulated in *Woohwangcheongsim-won* and *Sohaphyang-won*. This shows an upregulation of oxygen transporter under hypoxia, which means that these medicines have a significant effect in inhibiting apoptosis.

Myf6 is a myocyte differentiation gene and when muscles are created³⁶⁾, it is upregulated in the muscle

while its expression is limited at the mRNA level³⁷. In this study, Myf6 was downregulated in *Woohwangcheongsim-won* and *Sohaphyang-won*, but we could not explain the reason the myocyte related gene was expressed in the neuron. Also, it is difficult to find the reason for the downregulation of Slc12a1, which is a cotransporter of Na(+)-K(+)-2Cl(-)³⁸.

In this study, when expressed genes of *Woohwangcheongsim-won* and *Arisaema amurense* var. *serratum* N_{AKAI} were compared, Rar α was simultaneously upregulated [Global M = 1.0 (*Woohwangcheongsim-won*), 0.6 (*Arisaema amurense* var. *serratum* N_{AKAI})]. Rar α is a receptor in the retinoic acid that is derived from the activation of vitamin A. Retinoic acid is a necessary component in the creation of the nervous system and internal organs, and it is indispensable during the initial creation of the nervous system and the hindbrain³⁹. Based on the finding that Rar α was upregulated in *Woohwangcheongsim-won* and *Arisaema amurense* var. *serratum* N_{AKAI}, it appears that it protects the neurons under hypoxia.

When expressed genes of *Woohwangcheongsim-won* and *Coptis chinensis* F_{RANCH} were compared, Rab4a [Global M = -0.6 (*Woohwangcheongsim-won*), -0.6 (*Coptis chinensis* F_{RANCH})] and Pdgfra [Global M = -0.6 (*Woohwangcheongsim-won*), -1.3 (*Coptis chinensis* F_{RANCH})] were commonly downregulated. Early endosome is limited by the gradual activation of PKC and Rab4a, and Rab4a recycles gene endocytic⁴⁰. It is difficult to find a reasonable explanation for the downregulation of Rab4a in *Woohwangcheongsim-won* and *Coptis chinensis* F_{RANCH}. Pdgfr α is upregulated in medulloblastoma, and by boosting cell division, it creates undivided neuroblasts or neuroglia, thereby being involved in the generation of nerve tumors. The reason this gene is downregulated in *Woohwangcheongsim-won* and *Coptis chinensis* F_{RANCH} may be related to neuron protection, but it is difficult to explain in more

detail⁴¹.

When expressed genes of *Woohwangcheongsim-won* and *Rheum coreanum* N_{AKAI} were compared, phospholipase C and gamma 1 (Plcg γ 1) [Global M = 0.6 (*Woohwangcheongsim-won*), 0.8 (*Rheum coreanum* N_{AKAI})] were commonly upregulated, and phospholipase A2, group IB, pancreas (Pla2g1b) [Global M = -0.6 (*Woohwangcheongsim-won*), -0.7 (*Rheum coreanum* N_{AKAI})], and solute carrier family 10, member 1 (Slc10a1) [Global M = -0.8 (*Woohwangcheongsim-won*), -1.3 (*Rheum coreanum* N_{AKAI})] were commonly downregulated.

Plc γ 1 induces the creation of blood vessels, and the upregulation under hypoxia can be understood as a defense mechanism against cell damage⁴². Pla2g1b is synthesized in the acinar cell of the pancreas and then released in the intestine during food intake. It is known to accelerate obesity and diabetes by weight loss induced diet⁴³. Meanwhile, Slc10a1 is known to be the transporter of Na⁺ and bile acid⁴⁴, and it is difficult to explain its downregulation by *Woohwangcheongsim-won* and *Rheum coreanum* N_{AKAI}.

Thus is the comparison and analysis result of the medicines' characteristics using the clustering analysis method based on the experimental research that compared the gene expression of single herbal treatment and herbal medicines through the cDNA microarray. Despite limitations, through the analysis result of cDNA microarray, we could collect information related to the characteristics of genes commonly expressed between individual herbal treatments or medicines. If we continue the relations analysis research systematically with the herbal medicine mechanism, we hope to better understand the details of the mechanism and the role of herbal treatments and medicines in the future.

Conclusion

We found the following conclusions through the comparison and analysis results using the cDNA microarray analysis method and functional clustering method of gene expression alteration due to herbal treatments or single herbal medicines within the apoptosis induced neuron under hypoxia model.

1. Akt2 was upregulated by *Acorus gramineus* SOLAND, *Arisaema amurense* var. *serratum* NAKAI and *Coptis chinensis* FRANCH, and they belong to Araceae herb.
2. NF- κ b1, Cd5, Gn γ 7 and Sgne1 were upregulated by *Arisaema amurense* var. *serratum* NAKAI, *Coptis chinensis* FRANCH and *Rheum coreanum* NAKAI.
3. *Woohwangcheongsim-won*, *Sohaphyang-won* and *Scutellaria baicalensis* GEORGI downregulated Scp2, and upregulated Tsc2. *Woohwangcheongsim-won* and *Sohaphyang-won* upregulated Hba1, and downregulated Myf6.
4. *Sohaphyang-won* and *Scutellaria baicalensis* GEORGI downregulated Slc12a1.
5. *Woohwangcheongsim-won* and *Arisaema amurense* var. *serratum* NAKAI upregulated Rar α , *Woohwangcheongsim-won* and *Coptis chinensis* FRANCH downregulated Rab5a and Pdgfr α , and *Woohwangcheongsim-won* and *Rheum coreanum* NAKAI upregulated Plc γ 1 and downregulated Pla2g1b and Slc10a1.

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