Induction of heat shock protein (hsp 70) in human lymphocytes by Kampo herbal medicine

Megumi SUZUKI,[a,c,d] Guang-Bi JIN,[b] Meibo TOMII,[a]
Katsuji OGUCHI[c] and Jong-Chol CYONG[*a,d]

[a]*Department of Bioregulatory Function, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyou-Ku, Tokyo 113-8655, Japan.*
b)Department of Geriatrics, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyou-Ku, Tokyo 113-8655, Japan.
[c]*Department of Pharmacology, Showa University, School of Medicine, 1-5-8 Hatanodai, Shinagawa-Ku, Tokyo 142-8555, Japan.*
[d]*Medical History, Juntendo University , School of Medicine, 3-1-3 Hongo, Bunkyou-Ku, Tokyo 113-8431, Japan.*

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Abstract

Heat shock protein (hsp70) plays an important role in normalizing proteins that have degenerated due to stress, and in maintaining the physiological functions of cells. It has been reported that the induction of hsp70 may decline aging, and may be involved in autoimmune diseases and bacterial infection. In this study, we screened 233 kinds of extracts of Kampo herbal medicines on the expression of hsp 70 in human lymphocytes. Among them, 7 kinds of Kampo herbal medicine extracts, namely *Carthami Flos, Corni Fructus, Crataegi Fructus, Dianthi Herba, Euphorbiae Kansui Radix, Scutellariae Radix* and *Sojae Semen Praeparatum*, increased hsp70 expression one and half times in human lymphocytes. Among those, 6 kinds of extracts were confirmed to increase it in a dose-dependent manner. From these results, it was proved that some Kampo herbal medicines have the ability to control the expression of hsp70, and it was suggested that these herbs could be applicable for the treatment of various diseases in which the hsp system participates.

Key words heat shock protein (hsp) 70, human activated T lymphocyte, Kampo herbal medicine.
Abbreviations hsp, heat shock protein; PBS, phosphate- buffered saline ; IL-2, interleukin-2.

Introduction

Heat shock protein (hsp) is present in all organisms from *E. coli* to humans and has been preserved very well over species. Hsp is generally determined by its molecular weight and divided into four groups; around 90,000 molecular weight is classified as hsp90 family, around 70,000 as hsp70 family, around 60,000 as hsp60 family, and around 26,000 as low molecular weight hsp. Of these, hsp70 has an important role in normalizing proteins which have degenerated due to stress, and in maintaining the physiological functions of cell.[1] It has been reported that the induction of hsp70 may decline aging, and may be involved in autoimmune diseases and bacterial infection.[2,3]

We therefore made a hypothesis that an increase in the expression of hsp may have a favorable effect on these diseases. We have already reported that extracts of Kampo herbal medicines from among the 230 kinds have an effect of hsp70 induction on human neuroblastoma IMR-32 cells.[4] In this study, we measured the expression of hsp 70 in human lymphocytes, using 233 kinds of extracts of Kampo herbal medicines.

Materials and Methods

Human activated T lymphocytes: We took human

*To whom correspondence should be addressed. e-mail : cyong-tky@umin.ac.jp

peripheral blood (50 ml) with a syringe which contained 5 ml of heparin (heparin sodium injection Upjohn 1,000; Pharmacia, NJ, USA). Lymphocytes were layered on Ficoll-Conray solution (Lymphosepar I : specific gravity 1.077±0.001 ; IBL Co., Lab., Gunma, Japan), and separated by gradient differential centrifugation. The lymphocytes were washed in RPMI-1640 with L-Gln, $NaHCO_3$ medium (Nikken Bio Medical Laboratory, Kyoto, Japan). The bottom of the flask contained immobilized monoclonal antibody to CD3 (5 μg/ml;Janssen, Tokyo, Japan). In the flask, washed lymphocytes and RPMI-1640+7 with L-Gln, $NaHCO_3$ medium (Nikken Bio) were cultured (37℃, 5% CO_2 , humidity 98%). The medium was supplemented with 10% human serum (ICN Biomedicals Inc., Osaka, Japan) and interleukin-2 (IL-2:700 IU/ml;Shionogi Pharmaceuticals, Osaka, Japan). On day 3, we added the same medium for further culture, and on day 4, we again added the same medium with 30 mM HEPES (Sigma, MO, USA). On day 5, the lymphocytes were transferred to the backpack medium. The medium consisted of CP-4 medium (LL7.3 ; Nikken Bio) and AIM-V 101 medium (GIBCO BRL, MD, USA) with added IL-2 and oxalosuccinic acid (Sigma). Furthermore, these lymphocytes proliferated. These cultured lymphocytes were used as human activated T lymphocytes.

Preparation of Kampo herbal medicines and their extract for screening: The 233 kinds of Kampo herbal medicines were purchased from Uchida Wakanyaku Co. (205 kinds) (Tokyo, Japan) and Tochimoto Tenkaido (28 kinds) (Osaka, Japan). These herbal medicines were deposited at the Department of Bioregulatory Function, University of Tokyo, Japan. Each Kampo herbal medicine (15 g) was extracted with distilled water (300 ml) to half the original amount, and the supernatant was centrifuged (4℃, 3,500 rpm, 20 min). After aspirating filtration with filter paper, the solution was adjusted to 150 ml. Polychlal AT (Gokyosangyo, Japan) (1.5 g) was added to the extract (75 ml) and the mixture was shaken for 30 minutes at room temperature to eliminate tannic acid. After elimination, the solution was filtered with filter paper and adjusted to 75 ml. The solution (70 ml) was then lyophilized and the rest (5 ml) was used as Kampo herbal medicine extract for the first screening.

Measurement of hsp70: Human activated T lymphocytes (3×10^6 cell/dish) were infused in 24-well tissue culture plates to measure hsp70. Kampo herbal medicine extract diluted to 1 mg/ml was added to each plate and cultured (37℃, 5% CO_2, 18 hours). After heat shock for 40 minutes in a thermostat (Tomy, Japan) at 42℃, the cells were cultured (37℃, 5% CO_2, 3 hours) and collected. They were washed three times is PBS and frozen at -20℃ overnight. After thawing, the cell membranes were destroyed by homogenization (DIAX 100, Heidolph, Germany) for 2 minutes. Protein concentration in the solution was measured using Protein Assay (Bio-Rad Laboratories, CA, USA), and the amount of protein was regulated.

For protein assay of hsp70, 30 μl of each sample was placed into 96-well polystyrene ELISA plates (Sumitomo Bakelite Co., LTD., Tokyo, Japan), and 30 μl of coating buffer (20 mM carbonate buffer, pH 9.5) was added to each well. The assay plates were covered with a lid and incubated overnight at 4℃. The wells were washed with washing buffer (PBS with 1% Tween 20) and blocked by blocking buffer (PBS with 1% BSA). The plates were washed again, and 100 μl of alkaline phosphatase (AKP) conjugated anti-hsp70 monoclonal antibody (StreesGen Biotecnologies Corp, BC, Canada) diluted at 1:500 in blocking buffer was added to each well and the mixture incubated for 90 minutes at room temperature. After washing, 100 μl of p-nitrophenyl phosphate disodium (pNPP; Sigma) substrate (1 mg pNPP/ml in 1 M diethanolamine buffer containing 0.5 M $MgCl_2$, pH 9.8) was added. After incubation for 60 minutes at room temperature, absorbance was measured at 405 nm by a Microplate Reader (Bio-Rad model 550).

A standard curve was obtained using various concentrations (7.8-500 ng/ml) of recombinant human HSP70 protein (StressGen). The concentrations of hsp70 in samples were determined by standard curve and the results were converted to protein concentration.

Results

Result of the screening

In advance of screening, we performed two tests of the expression of hsp70. The two tests measured time-response (3, 6, 18 hours) after heat shock (42℃, 40 min) and the cell number of human activated T lymphocyte. The results confirmed that the expression of hsp70 was strongest when the number of cells was $3\sim4\times10^6$ cell/-well and the time of incubation was 3~6 hours after heat

Table I Effect of 233 extracts of Kampo herbal medicines on hsp 70 expression in human activated T lymphocytes

Kampo herbal medicines		human lymphocyte	IMR-32	Kampo herbal medicines		human lymphocyte	IMR-32
Achyranthis Radix	牛膝			Codonopsis pilosula Nannf	党参	▲	
Aconiti Radix	鳥頭			Coicis Semen	薏苡仁		○
Aconiti Tuber	附子			Corni Fructus	山茱萸	○	
Adenophorae Radix	北沙参			Cortidis Rhizoma	黄連		
Akebiae Caulis	通草			Corydalis Tuber	延胡索	▲	
Akebiae Caulis	木通			Crataegi Fructus	山楂子	○	
Alismatis Rhizoma	沢瀉			Crotonis Semen ★	巴豆		
Allii Folium	薤白	▲		Curcumae rhizoma	宇金		
Alpiniae Katsumadii Semen	草豆蔻			Cuscuta chinensis Lam	兎絲子		
Alpiniae Officnari Rhizoma	良姜			Cynanchi Atrati Radix	白薇		
Alpniae oxphyllae Fructus	益知			Cynomorii Herba	鎖陽		
Amomi Rotundi Fructus	白豆蔻			Cyperi Rhizoma	香附子		
Amomi Semen	縮砂			Desmodii Herba	金銭草		
Amomi Tsao-ko Fructus	草果			Dianthi Herba	瞿麦	○	
Amydae Carapax	別甲			Dioscoreae Rhizoma	山薬		
Anemarrhenae Rhizoma	知母			Dipsaci Radix ★	続断		
Angelicae Dahuricae Radix	白芷			Dolichoris Semen	白扁豆		
Angelicae Radix	当帰			Elsholtziae Herba	香需		
Aquilariae Lignum	沈香			Ephedrae Herba	麻黄		
Araliae Cardatae Rhizoma	独活			Epimedii Herba ★	淫羊霍		○
Arctii Fructus	牛蒡子			Equiseti Herba	木賊		
Arecae Pericarpium	大腹皮			Eriobotryae Folium	枇把葉		
Arecae Semen	檳榔子			Eucommiae Cortex	杜仲		
Arisaematis Tuber	天南星			Eupatori Herba ★	蘭草		
Armeniacae Semen	杏仁			Euphorbiae Kansui Radix	甘遂	○	
Artemisiae Folium	艾葉	▲		Euryales Semen	芡実		
Artemisoae Capillari Spicab	茵蔯蒿			Evodiae Fructus	呉茱萸	○	
Asiasari Radix	細辛			Farfarae Flos	款冬花		
Asini Corii Collas	阿膠			Foeniculi Fructus	小茴香		
Asparagi Radix	天門冬			Forsythiae Fructus	連翹		○
Asteris Radix Et Rhizoma	紫苑			Fossilia Ossis Mastodi	竜骨		
Atractylodis Lanceae Rhizoma	蒼朮			Fraxini Cortex ★	秦皮		
Atractylodis Rhizoma	白朮			Fritillariae Bulbus	貝母		
Aurantii Fructus Immaturus	枳実			Galla Rhois	五倍子		
Aurantii Nobilis Pericarpium	陳皮			Gambir Extractum	阿仙薬		
Bambusae Caulis	竹茹			Ganoderma	霊芝		○
Belamcandae Rhizoma	射干			Gardeniae Fructus	山梔子		○
Benincasae Semen	冬瓜子			Gastrodiae Tuber	天麻		
Biotae Orientalis Cacumen	側柏葉			Gentianae Macrophyllae Radix	秦艽		
Bletillae Tuber	白及			Gentianae Scabrae Radix	竜胆		○
Bombyx Batryticatus	白姜蚕			Ginseng Radix	人参		
Bupleuri Radix	柴胡			Gleditsiae Semen	皂角子		
Cannabis Fructus	麻子仁			Gleditsiae Spina	皂角刺		
Carthami Flos	紅花	○	○○	Glycyrrhizae Radix	炙甘草		
Caryophylli Flos ★	丁香			Haliotis gigantea discus Reeve	石決明		
Cassiae Torae Semen	決明子		○	Halloysitum rubrum	赤石脂		
Castanea crenata Fructus ★	栗			Hirudo	水蛭		
Chaenomelis Fructus	木瓜			Hoelen	茯苓		
Chebulae Semen	訶子			Hordei Fructus Germinatus	麦芽		
Chrysanthemi Flos	菊花			Houttuyniae Herba ★	魚腥草		
Cibotii Rhizoma	狗脊			Imperatae Rhizoma	茅根		
Cicadae Periostracum	蝉退			Ipomoea hederacea Jacq	牽牛子		○
Cinnamomi Cortex	桂皮			Junci Caulis Medulla	燈心草		
Cirsium japonica DC. ★	大小薊			Kaki Calyx	柿蒂		
Cistanchis Herba	肉従蓉			Kochiae Fructus	地膚子		
Citrus reticulata Blanco	青皮			Laminaria japonica ★	昆布		
Clematidis Radix	威霊仙			Leonuri Herba	益母草		
Cnidii Monnieri Fructus	蛇床子			Lepidii Semen	亭癈子		

Kampo herbal medicines		human lymphocyte	IMR-32	Kampo herbal medicines		human lymphocyte	IMR-32
Ligustri Semen	女貞子			Quercus Cortex ★	土骨皮		
Lilli Bulbus	百合			Quercus salicina Blume	裏白樫		
Linderae Radix	烏薬		○○	Rauwolfiae Radix ★	印度蛇木		○○
Lingustici Sinensis Rhizoma Et Radix	藁本			Rehmanniae Radix	地黄		
Lini Semen	亜麻仁			Rhei Rhizoma	大黄		
Lithospermi Radix	紫根			Rhus verniciflua Stokes ★	乾漆		
Longan Arillus	竜眼肉		○	Roasted Aconiti Tuber	炮附子		
Lonicerae Flos	金銀花			Rosae Laevigatae Semen	金桜子		○
Lonicerae Folium Cum Caulis	忍冬			Rubi Fructus	覆盆子		
Lophatheri Herba	竹葉			Rubiae Radix ★	茜草根		
Loranthi Ramulus	桑寄生			Saccharum Granorum	膠飴		
Lumbricus	地龍			Salviae Miltiorrhizae Radix	丹参		
Lycii Fructus	枸杞子			Sanguisorbae Radix	地楡		
Lycii Radicis Cortex	地骨皮			Saposhnikoviae Radix	防風		
Magnoliae Cortex	厚朴	▲		Sappan Lignum	蘇木		
Magnoliae Flos	辛夷			Saussureae Radix	木香		
Meliae Toosendan Semen ★	川楝子			Schisandrae Fructus	五味子		
Menthae Herba	薄荷	▲		Schizonepetae Spica	荊芥		
Mori Cortex	桑白皮			Scorophulariae Rasix	玄参		○
Mori Folium	桑葉			Scutellariae Herba	半枝連		
Morindae Radix	巴戟天			Scutellariae Radix	黄芩	○	○○
Moutan Crtex	牡丹皮			Sepiae Os ★	烏賊骨		
Mume Fructus	烏梅			Sesami Semen	胡麻		
Myrrha Resina ★	没薬			Sinapis Semen	白芥子		
Natrium Sulfuricum	芒硝			Sinomeni Caulis et Rhizoma	防已		
Nelumbis Semen	蓮肉		○	Smilacis Glabrae Rhizoma	山帰来		
Notopterygii Rhizoma	羌活			Sojae Semen Praeparatum	香鼓	○	
Nupharis Rhizoma	川骨			Sophorae Flos	槐花		○
Olibanum Resina	乳香			Sophorae Radix	苦参		
Ophiopgonis Tuber	麦門冬			Sophorae Subprostratae Radix	山豆根		
Oryzae Semen	粳米			Sparganii Rhizoma	三稜		○○
Ostreae Testa	牡蛎			Stellariae Dichotomae Radix	銀柴胡		
Paeoniae Radix	赤芍			Tabanus	虻虫		
Paeoniae Radix	芍薬		○	Talcum Crystallinum	滑石		
Panacis Japonici Rhizoma	竹節人参			Taraxacum platycarpum	蒲公英		
Patriniae Herba ★	敗醤草			Terra flava	黄土	▲	
Perillae Folium ★	紫蘇葉			Testudinis Plastrum	亀板		
Perillae Semen	紫蘇子			Thea sinensis ★	細茶		
Persicae Semen	桃仁			Thujae orientalis Semen	柏子仁		
Pharagmitis Rhizoma ★	芦根		○	Trachycarpi Folium et Petiolus	棕呂葉		
Phaseoli Semen ★	赤小豆		○	Trametes versicolor ★	カワラタケ		
Phellodendri Cortex	黄柏			Tribuli Fructus	蔓荊子		
Phytolaccae Radix ★	商陸			Trichosanthis Fructus	瓜呂実		
Picrorrhizae Rhizoma	胡黄連			Trichosanthis Radix	瓜呂根		
Pinctada martensii Duker ★	珍珠母			Trichosanthis Semen	瓜呂仁		
Pinelliae Tuber	半夏			Tritici Semen	小麦		
Plataginis Herba	車前草			Trogopterorum Faeces ★	五霊脂		
Plataginis Semen	車前子			Typhae Pollen	蒲黄		
Platycodi Radix	桔梗			Uncariae Uncis Cum Ramulus	釣藤鈎		
Pogostemi Herba	霍香	▲		Vespa Nidus	露峰房		○
Polygalae Radix	遠志		○	Vitexro tundifolia L.	蔓莉子		
Polygoni Multiflori Radix	何首烏			Xanthii Fructus	蒼耳子		
Polyporus	猪苓			Zanthoxyli Fructus	山椒		
Porites nigrescens Dany ★	海浮石			Zedoariae Rhizoma	莪朮	▲	○
Prunellae Spica	夏枯草		○○	Zingiberis Rhizoma	生姜		
Prunus salicina Cortex	李根皮			Zingiberis Siccatum Rhizoma	乾姜		
Psoraleae Semen	破胡紙			Zizyphi Fructus	大棗		
Puerariae Radix	葛根			Zizyphi Spinosi Semen	酸棗仁		
Pulsatillae Radix	白頭翁						

Human activated T lymphocytes were cultured for 18 hrs with Kampo herbal medicine extract diluted to 1 mg/ml and then subjected to heat shock (40 min, 42℃). Hsp70 expression was analyzed by ELISA. The result of IMR cells used reference 4. The round mark means an enhancing activity of herbal extracts on hsp 70 expression of more than 150% (○) and 200%(○○) compared to the control. A triangular mark means an activity of herbal extracts under 60% or less (▲) of hsp 70 expression as compared with control. Kampo herbal medicines marked by a star (★) were purchased from Tochimoto Tenkaido, and the others were from Uchida Wakanyaku.

shock. With this in mind, out of the 233 kinds of Kampo herbal medicine extracts, the 7 kinds which increased the amount of hsp70 by more than one and a half times were confirmed (Table I). The specimen numbers were *Carthami Flos* (Uchida 253026), *Corni Fructus* (Uchida 302723), *Crataegi Fructus* (Uchida 302817), *Dianthi Herba* (Uchida VMAMN), *Euphorbiae Kansui Radix* (Uchida USLNO), *Scutellariae Radix* (Uchida HO253109) and *Sojae Semen Praeparatum* (Uchida VMANO) (Fig 1). Compared with the controls, of these 7 kinds of Kampo herbal medicine extracts without heat shock, there was no significant difference except in the case of *Dianthi Herba*, which tended to increase the amount nearly one and half times. We confirmed the 6 kinds of Kampo herbal medicine extracts that suppressed the expression of hsp70 to less than three-fifths, compared with the controls. The specimen numbers were *Allii Folium* (Uchida VNBVP), *Artemisiae Folium* (Uchida 352606), *Codonpsis pilosula Nannf* (Uchida US192610), *Corydalis Tuber* (Uchida US142912), *Pogostemi Herba* (Uchida 142717), and *Zedoariae Rhizoma* (Uchida 252112). Without heat shock, each extract suppressed the expression of hsp70 compared with the control. With heat shock, all Kampo herbal medicine extracts except *Corydalis Tuber* showed a tendency to increase suppression of the expression of hsp70 (Data not shown).

Result of the dose response

We examined dose-dependency to confirm the reproducibility of the effect of the extracts that increased the amount of hsp expression by one and a half times or decreased it by three-fifths on the first screening (Fig.2). We confirmed the examinations in two concentrations (1, 10 mg/ml) because each of the Kampo herbal medicine extracts used on the first screening was a very small amount.

As a result, of the 7 kinds of Kampo herbal medicine extracts which increased the expression of hsp more than one and a half times, *Carthami Flos, Corni Fructus,*

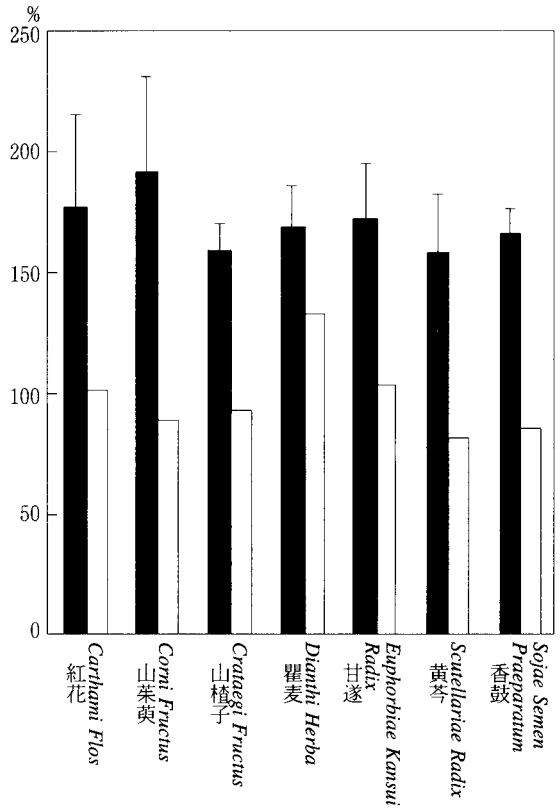

Fig. 1 Effect of Kampo herbal medicine extracts representing activity in the screening test on hsp70 expression in human activated T lymphocytes. Human activated T lymphocytes were cultured with the extracts (1 mg) for 18 hours prior to heat shock (■) (40 min. 42℃) or without heat shock (□). Hsp70 was measured by ELISA. Data are shown as mean±S.D. (n=3).

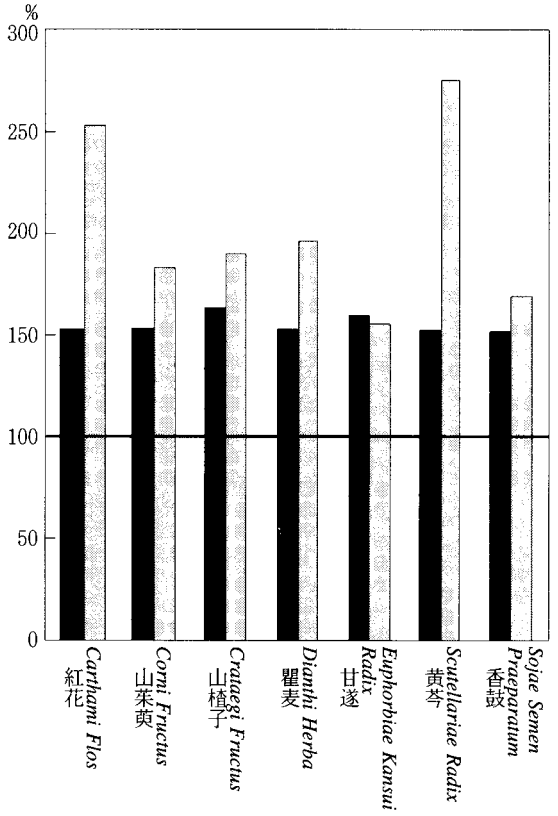

Fig. 2 Dose-dependency to confirm reproducibility concerning the 7 kinds of extracts. Human activated T lymphocytes were cultured with the extracts (1(■),10(□) mg) for 18 hours prior to heat shock (40 min. 42℃). Hsp70 was measured by ELISA.

Crataegi Fructus, Dianthi Herba, Scutellariae Radix and *Sojae Semen Praeparatum* were confirmed to increase it in a dose-dependent manner. Among these, *Carthami Flos* and *Scutellariae Radix* increased it by two and a half times at 10 mg/ml. *Euphorbaie Kansui Radix* increased it more than one and a half times in both concentrations (1, 10 mg/ml), but there was no dose-dependent increase. Of the 6 kinds of Kampo herbal medicine extracts which decreased the expression of hsp less than by three-fifths, *Allii Folium, Corydalis Tuber, Pogostemi Herba* and *Zedoariae Rhizoma* were confirmed to decrease it but not in a dose-dependent manner. 10 mg/ml of *Artemisiae Folium* and *Codonpsis pilosula Nannf* decreased it when compared with controls, but there was less increase in expression of hsp70 than with 1 mg/ml.

Discussion

The induction of hsp has been reported to decrease in the senile and to be involved in autoimmune diseases, cerebral ischemia and bacterial infection. That is to say, hsp is expressed when a cell is damaged, and at the same time functions to repair the damage. Therefore, there is a possibility that its mechanism could be used for the treatment of various diseases and for improvement of the quality of life in the aged, if we could control the expression of hsp. We therefore screened the 233 extracts of Kampo herbal medicines as a source of natural substances. Our results show that 7 kinds of Kampo herbal medicine extracts could increase the expression of hsp70, and that 6 kinds were confirmed to increase it in a dose-dependent manner. Out of the 6 kinds of Kampo herbal medicines (*Carthami Flos, Linderae Radix, Prunellae Spica, Rauwolfiae Radix, Scutellariae Radix,* and *Sparganii Rhizoma*) which were found to increase the expression of hsp70 more than twice from the results of the screening using human neuroblastoma IMR-32 cells last time,[4] 2 kinds (*Carthami Flos* and *Scutellariae Radix*) could also be confirmed to increase it in the screening using human activated T-lymphocytes. These two have common pharmacological actions such as antihypertensive, antitumor and immunopotentiated effects, and they are used clinically for the treatment of hypertension. We previously reported that stress might affect atherosclerosis and hypertension.[5] Furthermore, we presume that hsp may be associated with the pathogenesis of hypertension and thus with the effects of Kampo herbal medicines. Arachidonic acid is known as a substance promoting the induction of hsp 70. Promoting the turnover of arachidonic acid cascade is considered to influence the continuous activation of hsp in a mechanism promoting the induction of hsp 70.[6]

The 6 kinds of Kampo herbal medicine extracts could decrease the expression of hsp70. Galan *et al.* proved that the bacterial type Ⅲ secretion system is used as a means of infection by bacteria causing food poisoning by entry into the host cell cytosol, where they modulate cellular processes. Inhibiting the bond between secretory protein and cognate chaperon may possibly suppress the infection caused by various kinds of bacteria, and it may lead to a strategy developing a new treatment for infectious diseases.[3] It has been proved that induction of hsp is caused by activation at a transcription stage of RNA synthesis.[7,8] Quercetin, a bioflavonoid widely distributed in plants, has many biological effects, and inhibits the synthesis of hsp induced by heat shock and other stresses.[9,10] Quercetin inhibited the induction of hsp70 at the level of mRNA accumulation through inhibition of the activation of a heat shock transcription factor.[11] Some of the 6 kinds of Kampo herbal medicines contained tannin. These Kampo herbal medicines had the pharmacological actions of sedation and digestion. However, we are still trying to determine which constituent suppresses the expression of hsp.

A unique transcription factor has been proved to play an important role, even if details of molecular structure are different. Many intracellular actions are involved in a stress response; however, details such as which factor interact with which are not yet elucidated. At this time it appears that some Kampo herbal medicine extracts expressing hsp70 may be influenced by an activation of transcription, an activation of protein synthesis, or an increase in power of protein synthesis. We are now trying to identify substances influencing the expression of hsp 70. Further studies will be needed to determine substances governing the expression of hsp, and enable us to start developing a new treatment.

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和文抄録

ストレス蛋白質のうちhsp70は，ストレスにより変性した蛋白質を正常化し，細胞の生理機能維持のために重要な役割を果たしている。hsp70の誘導は，老化により低下することや，癌・自己免疫疾患・細菌感染などの病態に関与することが報告されている。今回，我々はヒト活性化T細胞を使用して，233種類の常用和漢薬について，hsp70の発現量のスクリーニングを行なった。スクリーニングの結果，1.5倍以上の増加傾向が認められた生薬は以下の7種類（紅花・山茱萸・山楂子・瞿麦・甘遂・黄芩・香鼓）であった。そのうち，濃度依存性が確認できた生薬は6種類であった。以上のことから，いくつかの生薬にhspの発現量をコントロールできる活性があることが証明されて，hsp系の関与する様々な疾患の治療などに応用できる可能性が示唆された。

*〒113-8431 東京都文京区本郷3-1-3
順天堂大学医学部医史学研究室 丁 宗鐵

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