行政院國家科學委員會專題研究計畫　成果報告

大蒜有機硫成分增加解毒酵素--CYP 2B1 與 Pi 屬麩甘氨酸硫轉移胺表現之研究(3/3)

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中文摘要

大蒜具有抗氧化、抗腫瘤、抗血小板凝集以及降血脂等功效，一直受到許多學者廣泛研究。本研究以初代肝細胞模式探討大蒜有機硫成分(DAS、DADS、DATS)對於調控解毒酵素-π屬穀胱甘　硫轉移酶(π form of glutathione S-transferase, GSTP)表現的作用機轉。結果顯示，除了 DAS 之外，DADS 和 DATS (50-200μM)皆以劑量關係誘發 GSTP 蛋白質表現，在 200μM DADS 和 DATS 處理下，GSTP 蛋白質表現相較對照組(0.1% DMSO)分別增加 5.1 倍和 6.5 倍；mRNA 分析(Northern blotting)結果亦與蛋白質類似，DADS 和 DATS 皆以劑量關係誘發 GSTP mRNA 表現，且 DATS≧DADS＞DAS；在 GSTP 酵素活性方面，DAS 與對照組並無差異，但 DADS、DATS 亦以劑量依賴方式誘發GSTP 酵素活性表現。將帶有不等長度 GSTP 5'端啟動區的報導質體以 lipofectamine 方式轉殖至 clone 9 肝細胞株，Luciferase 活性分析顯示，DAS 其 luciferase 活性皆與控制組無差異；DADS 和 DATS 的 Luciferase 活性相較控制組分別增加 2.8 和 3.9 倍，若將位於 5'端啟動區-2.7 至-2.6 kb 的 GSTP enhancer I (GPE I)刪除則會破壞DADS 和 DATS 所誘發的 Luciferase 活性，然而將 GPE II 刪除則不影響，表示 GSTP-enhancer I (GPE I)是 DADS、DATS 增加 luciferase 活性所必需，對於 DADS、DATS 誘發 GSTP 基因表現扮演重要的角色(Journal of Nutrition, 2005)。第三年我們進一步針對訊號傳遞途徑，探討DADS 和 DATS 如何調節 GPE I，誘發 GSTP 表現；Electrophoretic mobility shift assay (EMSA)顯示，DADS 及 DATS 均可增加 AP-1 活化，SB600125 (JNK 抑制劑)和 PD98059 (ERK 抑制劑)則可抑制 AP-1 活化；MAPK 訊號途徑中，JNK 和 ERK 磷酸化因 DADS 和 DATS 處理呈現劑量增加，而預處理 SP600125 或 PD98059 則抑制磷酸化的發生；相較JNK 和 ERK，p38 磷酸化並不因 DADS 和 DATS 處理而變化。以上結果可知，DADS 和 DATS 這兩種脂溶性大蒜硫成分上調 GSTP mRNA 和蛋白質表現，可能係藉由 MAPK 訊號途徑中 JNK 和 ERK 活化 AP-1，進而與 GPE I 的 AP-1 結合區結合所致。

關鍵字:初代肝細胞、大蒜有機硫成分、π屬穀胱甘　硫轉移酶、基因轉殖

Part I: (accepted by the Journal of Nutrition)

Garlic Organosulfur Compounds Up-Regulate the Expression of the π Class of Glutathione *S*-Transferase in Rat Primary Hepatocytes

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[3] Abbreviations used: AP-1, activator protein-1; ARE, antioxidant response element; t-BHQ, tert-butylhydroquinone; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; DMSO, dimethylsulfoxide; GST, glutathione S-transferase; GSTP, π class of GST; GPEI, GSTP enhancer I; GPEII, GSTP enhancer II; TRE, 12-*O*-tetradecanoylphorbol-13-acetate response element.

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ABSTRACT The chemopreventive property of garlic is known to be partially related to its induction of phase II detoxification enzymes. In the present study, we investigated the modulatory effect of three garlic organosulfur compounds—diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS), which differ in their number of sulfur atoms—on the gene expression of the π class of glutathione *S*-transferase (GSTP). Hepatocytes isolated from male Sprague-Dawley rats were cultured with 50-200 μmol/L of DAS, DADS, or DATS for 24 h. The results showed that DADS and DATS significantly increased GST activity toward ethacrynic acid by 40% and 66%, respectively ($P<0.05$). Moreover, both garlic allyl sulfides dose-dependently induced GSTP mRNA and protein expression and the induction of protein level was in the order of DATS > DADS ($P<0.05$). In contrast, DAS had no significant effect on enzyme activity or the protein or mRNA levels of this phase II drug-metabolizing enzyme. In Clone 9 liver cells, the pTA-luciferase reporter assay showed luciferase activity in DADS- and DATS-treated cells to be 2.8- and 3.9-fold higher than that in control cells, respectively ($P<0.05$). Again, luciferase activity was not significantly changed by treatment with DAS. Deletion of -2.7 to -2.6 kb in the GSTP promoter region, which contains the GSTP enhancer (GPE) I element, abolished the up-regulation of GSTP transcription by DADS and DATS. Deletion of GPE II, however, did not affect the induction of reporter activity. In conclusion, the effectiveness of three garlic allyl sulfides on GSTP expression was related to the number of sulfur atoms in the molecules, and GPE I was responsible for such up-regulation.
KEY WORDS: *Garlic organosulfur compounds; π class of glutathione S-transferase; gene expression; hepatocytes; rats*

INTRODUCTION

Cancer chemoprevention includes the prevention, inhibition, or reversion of the process of carcinogenesis. Drug-metabolizing systems are composed of phase I and phase II enzymes. Phase I enzymes, mainly cytochrome P450, detoxify a variety of endogenous and exogenous chemicals and activate many carcinogens (1). Phase II enzyme systems, which include glutathione *S*-transferase (GST)[3], quinone reductase, sulfotransferases, and UDP-glucuronosyltransferase, catalyze the reduction or conjugation of phase I metabolites to various water-soluble molecules and accelerate the metabolite excretion rate. Higher tissue levels of phase II detoxification enzymes result in lower susceptibility to carcinogen insult (2,3).

GST catalyzes the conjugation of glutathione with a variety of electrophilic xenobiotics and facilitates their excretion. GST is composed of six distinct gene families, including five cytosolic groups (α, μ, π, θ, and σ) and one microsomal form (κ) (4). The different gene families share a similar function but differ in their substrate specificity. The class μ and θ GSTs are the major isozymes that participate in glutathione conjugation with benz[*a*]anthracene epoxides and polycyclic aromatic hydrocarbons (5). Recently, interest has been growing in the physiologic properties of the π class of GST (GSTP), not only because of its action in drug detoxification but also because of its possible roles in cell transformation (6,7). Compared with other isozymes, GSTP is more effective in the detoxification of electrophilic α,β-unsaturated carbonyl compounds that are generated by radical reactions of lipids (8). Because it is highly inducible during carcinogenesis, GSTP expression is regarded as an important determinant of cancer susceptibility and a reliable marker of tumorigenesis (9).

Two enhancing elements were identified in the 5' upstream region of the GSTP gene: GSTP enhancer I (GPEI, -2.5 kb) and GSTP enhancer II (GPEII, -2.2 kb) (10). The highly inducible characteristic of GSTP is generally attributed to GPEI (10), although GPEII contains the enhancer core-like sequence of simian virus 40. GPEI has two 12-*O*-tetradecanoylphorbol-13-acetate response-like elements (TREs), which are regulated by multiple factors, including activator protein-1 (AP-1) (11). Both TREs are thought to be required for the basal and inducible expression of GSTP (12,13).

Garlic displays diverse biological activities, including antithrombotic, antiatherosclerotic, antidiabetic, and antioxidant activities and immune modulation (14-17). Moreover, epidemiologic evidence shows that persons who consume large amounts of garlic (*Allium sativum* L.) in their diet have a decreased risk of stomach and colon cancers (18). The rich content of the numerous organosulfur compounds in garlic is well known to be key in garlic's health-related functions (19). Among the garlic organosulfur compounds, diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS), which differ in their number of sulfur atoms, are three major volatile allyl sulfides in garlic oil (20). The effectiveness of DAS, DADS, and DATS on the transcriptional regulation of phase I and phase II detoxification enzyme expression has been shown to be positively associated with their suppression of aflatoxin B_1- and benzo(*a*)pyrene–induced liver and forestomach neoplastic formation in mice and rats (21,22). Recently, DAS, DADS, and DATS were further shown to display differential effects in the decrease in cyclin-dependent kinase-Cdk7 and the increase in cyclin B1 protein levels in J5 human liver tumor cells and, thus, to arrest cells in the G2/M phase (23).

Structure-function relationship study has indicated that the biological potency of the garlic allyl sulfides is related to their number of sulfur atoms or allyl or propyl groups (22,24). Recently, in an animal study, we reported that the induction of GSTP protein and mRNA levels in rat liver by DAS, DADS, and DATS was in the order of DATS $\geqq$ DADS $>$ DAS (24). However, the trend was not always like this and depends on the target enzyme tested. For example, the inducibility of cytochrome P450 1A1, 2B1, and 3A1 expression was negatively related to the number of sulfur atoms and was in the order of DAS>DADS>DATS (24). This discrepancy suggests that the regulatory mechanism of the three garlic organosulfur compounds on GSTP and cytochrome P450 differs and is worthy of further study.

Although it is well established that garlic organosulfur compounds induce GSTP activity and protein expression, the molecular mechanisms of this up-regulation have not yet been studied. In the present study, we first examine the efficacy of DAS, DADS, and DATS on GSTP mRNA and protein expression in rat primary hepatocytes and then construct a reporter gene to identify whether the promoter regions of the GSTP gene contain an element responsible for the up-regulation of expression by allyl sulfides.

MATERIALS AND METHODS

Materials. DAS, DADS, and DATS were purchased from Fluka Chemical Co. (Buchs, Switzerland), Tokyo Kasei Chemical Co. (Tokyo, Japan), and LKT Laboratories (St. Paul, MN). ITS$^+$ was obtained from BD Biosciences (Bedford, MA). Ethacrynic acid, dexamethasone, and HEPES were obtained from Sigma Chemical Co. (St. Louis, MO). RPMI-1640 media, fetal bovine serum, and penicillin-streptomycin solution were obtained from Gibco Laboratory (Grand Island, NY). Trizol and lipofectamine were ordered from Invitrogen (Carisbad, CA).

Cell isolation and culture. Male Sprague-Dawley rats were purchased from the National Laboratory Animal Center (Taipei, Taiwan) and were used for hepatocyte isolation when aged 7 to 8 wk old. Rats were treated in compliance with the *Guide for the Care and Use of Laboratory Animals* (25). Hepatocytes were isolated by a two-step collagenase perfusion method as described previously (26). Cell viability was >90% as determined by trypan blue exclusion. The isolated hepatocytes were suspended in RPMI-1640 medium containing 10 mmol/L HEPES, 1×10^5 Unit/L penicillin, 100 mg/L streptomycin, 0.1 mmol/L dexamethasone, and 1% ITS. The cells were plated on 60-mm plastic tissue culture dishes (Nuck, Roskilde, Denmark) precoated with rat tail collagen VII at a density of 3×10^6 cells per dish and were incubated at 37^{o}C in a humidified atmosphere of 5% CO_2 and 95% air. Cell attachment on the culture dish was achieved 48 h after plating; fresh culture media containing various concentrations of DAS, DADS, or DATS was then added for an additional 24 h. Cells treated with 0.1% dimethylsulfoxide (DMSO) alone were regarded as controls.

SDS-polyacrylamide gel electrophoresis and immunodetection. Cells were washed twice with cold PBS and were harvested in 500 μL of 20 mmol/L potassium phosphate buffer (pH 7.0). Cell homogenates were centrifuged at 10,000 × *g* for 30 min at 4^{o}C. The resultant supernatant portion was then ultracentrifuged at 105,000 × *g* for an additional 1 h. Protein content was measured by using the Coomassie Plus Protein Assay Reagent Kit (Pierce Chemical Company, Rockford, IL). Equal amounts of cytosolic proteins from each sample were applied to 10% SDS-polyacrylamide gels and electrophoretically transferred to polyvinylidene fluoride membranes. The nonspecific binding sites on the membranes were blocked at 4°C overnight with 50 g/L nonfat dry milk in 25 mmol/L Tris/150 mmol/L NaCl buffer, pH 7.4. The blots were then incubated sequentially with primary antibodies against GSTP (Transduction Laboratories, Lexington, KY) or actin (Sigma Chemical, St. Louis, MO). After incubation with the horseradish peroxidase–conjugated secondary antibody, color was developed by adding hydrogen peroxide and tetrahydrochloride diaminobenzidine as peroxidase substrates.

Northern blot analysis. Total RNA was extracted with Trizol reagent. The cDNA probe was prepared by RT-PCR as described previously (27). One pair of oligonucleotide primers (forward: 5'-TTCAAGGCTCGCTCAAGTCCAC-3'; reverse: 5'-CTTGATCTTGGGGCGGGCACTG-3') was designed on the basis of the published sequences of GSTP (27,28). The band corresponding to the DNA fragment of GSTP was labeled with α-^{32}P-dCTP with the use of a NEBlot kit (New England Biolabs, Beverly, MA) and was used as the probe. For Northern blot analysis, the RNA sample was electrophoretically separated on an agarose gel and transferred to a HyBond N$^+$ membrane (Amersham, Little Chalfont, UK). The membrane was then prehybridized and hybridized as described (27). Autoradiography was performed by exposing the membrane to

Kodak SuperRx X-ray film at -80°C with an intensifying screen. The band intensity on the X-ray film was quantitated with an AlphaImager 2000 (Alpha Innotech, San Leandro, CA).

Expression and reporter constructs. The rat GSTP promoter region was generated by PCR amplification with rat genomic DNA as a template. The oligonucleotide primer (forward: 5'-GCCTCAGCTGGTAAATGGATAA-3'; reverse: 5'-AAAGGCCCCAGAGCCGCCA-GCC-3') was designed on the basis of the published sequence (10,29). The PCR reactions were performed as follows: 5 min at 94°C; 35 cycles of 40 s at 94°C, 40 s at 60°C, and 120 s at 72°C; and a final extension of 5 min at 68°C. The PCR amplicons were then electrophoresed in 1%-agarose gels containing 1X TAE buffer (40 mmol/L Tris, 20 mmol/L glacial acetic acid, and 2 mmol/L EDTA). The band corresponding to the designated length was excised, and the DNA was purified by using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA). DNA products were ligated to the pCR 2.1-TOPO vector according to the manufacturer's instructions (Invitrogen, Carlsbad, CA) for amplifying and sequencing (Mission Biotech, Taiwan). The fragment containing -1 to -2713 bp of the GSTP gene promoter was identified. For the pTA-GSTP Luc construct, the recombinant was subcloned into a pTA-SEAP/Luc vector (Clontech, Palo Alto, CA). In addition to the full-length construct (pTA-2713), two constructs with deletions from -2713 to ~-2605 bp (pTA-2604) and from -2713 to ~-2376 bp (pTA-2375) were generated.

Transient transfection and Luciferase activity assay. Clone 9 cells, which derived from normal rat livers, were obtained from Bioresources Collection and Research Center (BCRC, Taiwan). They were grown in RPMI-1640 medium supplemented with 10 mmol/L HEPES, 1×10^5 Unit/L penicillin, 100 mg/L streptomycin, and 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. For all studies, cells with passages between 4 and 10 were used. A total of 2×10^5 cells were plated on each 35-mm plastic tissue culture dish (Nuck, Roskilde, Denmark), and the dishes were incubated until 70% confluence was reached. Cells were transiently transfected for 4 h with 0.1 μg of the pTA-GSTP Luc vectors by lipofectamine reagent and were then changed to fresh culture media for 5 h before being exposed to DAS, DADS, DATS, or tert-butylhydroquinone (t-BHQ) for an additional 12 h. Cells were then washed twice with PBS and lysed in 100 μL of lysis buffer (Clontech, Palo Alto, CA). Luciferase activity was measured by using Luciferase Assay Reagent (Clontech, Palo Alto, CA) according to the manufacturer's instructions. The luciferase activity of each sample was corrected on the basis of ß-galactosidase activity, which was measured at 420 nm with *O*-nitrophenyl ß-D-galactopyranoside as a substrate. The value for cells treated with DMSO vehicle alone was regarded as 1.

Biochemical assays. GST activity was measured according to the method of Habig et al. (30) by using ethacrynic acid as the substrate because of its better selectivity for the π class (31). Briefly, the reaction mixture in a final volume of 1 mL contained 100 mmol/L potassium phosphate buffer (pH 6.5), 0.25 mmol/L glutathione, 0.2 mmol/L ethacrynic acid, and an appropriate amount of the cytosolic proteins. The ethacrynate-glutathione conjugate formed was measured at 270 nm.

Statistical analysis. Statistical analysis was performed with commercially available software (SAS Institute Inc, Cary, NC). Data were analyzed by means of one-way ANOVA, and

the significant difference among treatment means was assessed by use of Tukey's test. A value of $P<0.05$ was considered to be significant.

RESULTS

Allyl sulfides and GSTP protein level. The immunoblot assay showed that DADS and DATS dose-dependently increased GSTP expression in primary hepatocytes, and the extent of the increase caused by the two allyl sulfides was similar (**Fig. 1**A**)**. Treatment with DADS and DATS at a concentration of 200 μmol/L caused a 5.1- and 6.5-fold increase in the GSTP protein level, respectively, compared with that of the control cells (Fig. 1B). In contrast, DAS did not significantly affect GSTP protein expression.

GSTP mRNA level and enzyme activity. By Northern blot, the changes in GSTP mRNA levels were consistent with those noted for protein expression (**Fig. 2**). There was a dose-dependent induction of GSTP mRNA in cells treated with DADS and DATS but not with DAS. We additionally used ethacrynic acid as a substrate to measure GSTP activity. Compared with that in the control cells, enzyme activity was dose-dependently increased by treating the hepatocytes with 50 to 200 μmol/L of DADS and DATS ($P<0.05$) (**Fig. 3**). At the same concentrations, DAS had no significant effect on enzyme activity.

GSTP promoter activity. A cell viability assay, performed by the 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) method, showed that up to 200 μmol/L of each allyl sulfide was not toxic to the Clone 9 cells (data not shown). The response of GSTP in Clone 9 cells to each of the three garlic allyl sulfides was examined. These results indicated that the extent and pattern of GSTP protein expression were similar to that noted in rat primary hepatocytes (**Fig. 4**A). No significant change was noted in cells treated with DAS. However, a 2-fold increase in the GSTP protein level was induced by either DADS or DATS at a concentration of 50 μmol/L.

Three pTA-GSTP luciferase reporters containing a different length of the promoter region were constructed. The pTA-2713 reporter construct included both GPE I and GPE II, whereas the pTA-2604 construct included only GPE II. The third construct, pTA-2375, contained neither enhancer. The constructs were transiently transfected into Clone 9 cells to examine whether the promoter activity of the GSTP gene was modulated by the garlic allyl sulfides and to locate the possible responsive sites. When we transfected the pTA-2713 Luc reporter into the Clone 9 cells, we noted a dose-dependent increase in luciferase activity in the cells treated with DADS and DATS (Fig. 4B). At a concentration of 50 μmol/L, DADS and DATS caused a 1.0- and 1.9-fold increase in activity, respectively, compared with that in the control cells. In contrast, luciferase activity was not significantly changed by DAS. The increase in luciferase activity induced by 50 μmol/L DADS was similar to that induced by t-BHQ, an antioxidant and also a well-known phase II detoxification enzyme inducer. This induction of reporter activity was completely abolished when the -2713 to -2604 bp region of the GSTP promoter was deleted (Fig. 4C). The induction of reporter activity was also abolished in cells transfected with the pTA-2375 reporter construct. These results indicate that the sequences between -2713 bp and -2604 bp of the promoter region are responsible for the up-regulation of GSTP transcription by DADS and DATS.

DISCUSSION

The expression of GSTP is highly inducible not only by a variety of exogenous xenobiotics but also by several dietary factors, including nutrient and nonnutrient factors (24,27,32). Fish oil is one of the dietary factors that are effective in the up-regulation of this phase II detoxification enzyme (27). More recently, we further showed that methionine and cysteine restriction enhances GSTP expression in primary rat hepatocytes (32). Numerous garlic organosulfur compounds are also known to be effective inducers of GSTP (24). However, the actual molecular mechanism by which these dietary factors affect the transcriptional stage of GSTP expression had not previously been clearly elucidated. The results of the present study indicate that three lipid-soluble allyl sulfides, DAS, DADS, and DATS, differentially up-regulate GSTP expression in rat primary hepatocytes. By use of a pTA-GSTP luciferase reporter assay, we further showed than an enhancer element named GPE I located at -2.7 to -2.6 kb of the promoter region is responsible for the up-regulation of GSTP expression by DADS and DATS.

The results of epidemiologic studies have shown that garlic consumption is inversely correlated with the incidence of certain types of cancers (18). In animal studies, garlic allyl sulfides including DAS and DADS have been shown to be effective against aflatoxin B1- and azoxymethane-induced liver and colon tumorigenesis (21,33). Such an anti-tumorigenic effect is attributed to their modulation of phase II detoxification enzyme activity, which facilitates carcinogen clearance. Indeed, DAS and DATS have been shown to increase benzo(*a*)pyrene excretion by up-regulating GSTP expression (22). The importance of GSTP in cancer prevention is supported by the fact that 7,12-dimethylbenzanthracene-induced skin cancer was significantly elevated in GSTP-null mice (34). The effectiveness of DADS and DATS in increasing GSTP activity and expression suggests the potential application of these garlic allyl sulfides in chemoprevention (35,36).

Structure-function relationship study has been widely used to examine relative biological activity among structurally related phytochemicals (22,37). In the present study, the number of sulfur atoms in DAS, DADS, and DATS was shown to be correlated with the potency of these compounds in up-regulating GSTP gene expression. With a higher number of sulfur atom, GSTP mRNA and protein levels and enzyme activity were greater (Figs. 1-3). This result is consistent with our previous animal study (24). It is of interest to understand how the number of sulfur atoms in the garlic allyl sulfides differentially regulates GSTP gene expression. To our knowledge, no explanation for this effect exits, and further study will be necessary to solve this puzzle. We think that DAS, DADS, and DATS may display differential activation of a specific transcriptional factor or factors, of binding to the cis-acting element on the GSTP gene promoter, and, finally, of GSTP gene transcription. In addition, the differential transportation or detoxification rate of three allyl sulfides in liver cells may also be one of the possible explanations.

Clone 9 liver cells, which are a permanently growing, nontransformed rat liver cell line (38), are derived from normal rat liver and retain an epithelial morphology (39,40). The cell line has been used extensively as a model for hepatocyte function, including study of the mediation of expression of GSTP (41,42). By constructing Luc-reporters through serial deletion of the 5'-flanking region of the GSTP gene, we clearly showed that the section from -2713 bp to -2604 bp, which contains an enhancing element named GPE I, is required for the inducibility of GSTP

expression by DADS and DATS in Clone 9 cells (Fig. 4B and C). The second enhancing element, GPE II, which is located at -2.2 kb (10), however, had no influence on induction of the GSTP gene. This findings agrees with the reports by others that the highly inducible characteristic of GSTP is attributed mostly to GPE I and not to GPE II (10).

In the present study, GPE I and II were identified to be located 2.7 and 2.4 kb [2.5 and 2.2 kb in the report by Sakai et al. (10)] upstream of the transcription start site and to be about 0.2 kb longer than reported by Sakai et al. The entire 2.7-kb promoter was sequenced, and the nucleotide sequences of both the 3' [-398 ~ -1 bp in this paper and Okuda et al. (29)] and the 5' [-2713~-2376 bp vs. -2487 ~ -2150 bp in Sakai et al. (10)] ends were shown to be more than 99% identical to the published sequences (10,29). GPE I contains two TRE-like elements, and both elements are required for the basal and inducible expression of GSTP (12,13). Deletion of the TRE abolishes the induction of GSTP transcription by 3,4,5,3',4'-penta-chlorinated biphenyl in primary hepatocytes (12). Several transcriptional factors, particularly AP-1, have been shown to participate in the transcriptional activation of the enhancer of the GSTP gene (43). AP-1 motifs commonly compose either Jun homodimers or Jun/Fos heterodimers. It is still unclear whether Jun/Fos forms complexes with GPE I in the presence of DADS and DATS. Because of the existence of an AP-1-like binding site in the TRE, it is likely AP-1 plays an important role in the up-regulation of GSTP expression by DADS and DATS. Recently, extracellular-signal regulated kinase (ERK) and c-jun N-terminal kinase (JNK), two upstream regulators of AP-1, have been reported to be activated by DATS, and this activation is responsible for the DATS-induced apoptosis of human PC-3 prostate cancer cells (44). AP-1 mediates a wide variety of genes involved in various biological processes in cell proliferation, differentiation, transformation, apoptosis, inflammation, and immune responses (45). An understanding of the role of the AP-1-mediated signal pathway in GSTP transcriptional regulation will help to clarify the possible molecular mechanism of action of the active garlic components in drug metabolism and cancer prevention (44,46).

In addition to AP-1, Nrf2 is a possible transcriptional factor that may up-regulate GSTP expression because the TRE-like sequences on GPE I (5'-AGTCAGTCACTATGATTCAG-CA-3') share characteristics similar to those of the antioxidant response element (ARE, 5'-GTGACTTGGCA-3'). Nrf2 forms a heterodimer with small Maf and its binding to the ARE is known to be responsible for the induction of the α class of GST and other phase II detoxification enzymes, such as GSTP and NAD(P)H:quinone oxidoreductase (47). There is evidence that Nrf2/ARE pathway is also important in the DADS and DATS up-regulation of NAD(P)H:quinone oxidoreductase 1 and heme oxygenase (48). GSTP induction by 6-methylsulfinylhexyl isothiocyanate of wasabi was shown to be completely abrogated in Nrf2-deficient mice, which further suggests that the Nrf2/ARE pathway is likely involved in the 6-methylsulfinylhexyl isothiocyanate induction of this phase II detoxification enzyme (49). These raise the possibility that the up-regulation of GSTP expression by DADS and DATS may also be dependent on Nrf2. To solve this puzzle, further study is warranted.

In conclusion, garlic allyl sulfides differentially up-regulate GSTP mRNA and protein expression, and their effectiveness is related to their number of sulfur atoms. Moreover, GPEI, an enhancer element located at -2.7 kb, is required for the induction of this phase II detoxification enzyme.

LITERATURE CITED

1. Guengerich, F. P. & Shimada, T. Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem Res Toxicol. 1991; 4: 391-407.

2. Talalay, P., Fahey, J. W., Holtzclaw, W. D., Prestera, T. & Zhang, Y. Chemoprotection against cancer by phase 2 enzyme induction. Toxicol Lett. 1995; 82-83: 173-179.

3. Kensler, T. W. Chemoprevention by inducers of carcinogen detoxication enzymes. Environ Health Perspect. 1997; 105: 965-970.

4. van Iersel, M. L., Verhagen, H. & van Bladeren, P. J. The role of biotransformation in dietary (anti)carcinogenesis. Mutat Res. 1999; 443: 259-270.

5. Dusinska, M., Ficek, A., Horska, A., Raslova, K., Petrovska, H., Vallova, B., Drlickova, M., Wood, S. G., Stupakova, A. et al. Glutathione S-transferase polymorphisms influence the level of oxidative DNA damage and antioxidant protection in humans. Mutat Res. 2001; 482: 47-55.

6. Satoh, K., Kitahara, A., Soma, Y., Inaba, Y., Hatayama, I. & Sato, K. Purification, induction, and distribution of placental glutathione transferase: a new marker enzyme for preneoplastic cells in the rat chemical hepatocarcinogenesis. Proc Natl Acad Sci U S A. 1985; 82: 3964-3968.

7. Tsuchida, S. & Sato, K. Glutathione transferases and cancer. Crit Rev Biochem Mol Biol. 1992; 27: 337-384.

8. Berhane, K., Widersten, M., Engstrom, A., Kozarich, J. W. & Mannervik, B. Detoxication of base propenals and other alpha, beta-unsaturated aldehyde products of radical reactions and lipid peroxidation by human glutathione transferases. Proc Natl Acad Sci U S A. 1994; 91: 1480-1484.

9. Hu, X., Benson, P. J., Srivastava, S. K., Xia, H., Bleicher, R. J., Zaren, H. A., Awasthi, S., Awasthi, Y. C. & Singh, S. V. Induction of glutathione S-transferase pi as a bioassay for the evaluation of potency of inhibitors of benzo(a)pyrene-induced cancer in a murine model. Int J Cancer. 1997; 73: 897-902.

10. Sakai, M., Okuda, A. & Muramatsu, M. Multiple regulatory elements and phorbol 12-O-tetradecanoate 13-acetate responsiveness of the rat placental glutathione transferase gene. Proc Natl Acad Sci U S A. 1988; 85: 9456-9460.

11. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. Cell. 1987; 49: 729-739.

12. Matsumoto, M., Imagawa, M. & Aoki, Y. Identification of an enhancer element of class Pi glutathione S-transferase gene required for expression by a co-planar polychlorinated biphenyl. Biochem J. 1999; 338: 599-605.

13. Okuda, A., Imagawa, M., Maeda, Y., Sakai, M. & Muramatsu, M. Structural and functional analysis of an enhancer GPEI having a phorbol 12-O-tetradecanoate 13-acetate responsive element-like sequence found in the rat glutathione transferase P gene. J Biol Chem. 1989; 264: 16919-16926.

14. Peleg, A., Hershcovici, T., Lipa, R., Anbar, R., Redler, M. & Beigel, Y. Effect of garlic on lipid profile and psychopathologic parameters in people with mild to moderate hypercholesterolemia. Isr Med Assoc J. 2003; 5: 637-640.

15. Thomson, M. & Ali, M. Garlic [Allium sativum]: a review of its potential use as an anti-cancer agent. Curr Cancer Drug Targets. 2003; 3: 67-81.

16. Ou, C. C., Tsao, S. M., Lin, M. C. & Yin, M. C. Protective action on human LDL against oxidation and glycation by four organosulfur compounds derived from garlic. Lipids. 2003; 38: 219-224.

17. Lamm, D. L. & Riggs, D. R. Enhanced immunocompetence by garlic: role in bladder cancer and other malignancies. J Nutr. 2001; 131: 1067S-1070S.

18. Fleischauer, A. T. & Arab, L. Garlic and cancer: a critical review of the epidemiologic literature. J. Nutr. 2001; 131: 1032S-1040S.

19. Le Bon, A. M. & Siess, M. H. Organosulfur compounds from Allium and the chemoprevention of cancer. Drug Metabol Drug Interact. 2000; 17: 51-79.

20. Yu, T. H., Wu, C. M., and Liou, Y. C. Volatile compounds from garlic. J. Agric. Food Chem. 1989; 37: 725-730.

21. Guyonnet, D., Belloir, C., Suschetet, M., Siess, M. H. & Le Bon, A. M. Mechanisms of protection against aflatoxin B(1) genotoxicity in rats treated by organosulfur compounds from garlic. Carcinogenesis. 2002; 23: 1335-1341.

22. Hu, X., Benson, P. J., Srivastava, S. K., Mack, L. M., Xia, H., Gupta, V., Zaren, H. A. & Singh, S. V. Glutathione S-transferases of female A/J mouse liver and forestomach and their differential induction by anti-carcinogenic organosulfides from garlic. Arch Biochem Biophys. 1996; 336: 199-214.

23. Wu, C. C., Chung, J. G., Tsai, S. J., Yang, J. H. & Sheen, L. Y. Differential effects of allyl sulfides from garlic essential oil on cell cycle regulation in human liver tumor cells. Food Chem Toxicol. 2004; 42: 1937-1947.

24. Wu, C. C., Sheen, L. Y., Chen, H. W., Kuo, W. W., Tsai, S. J. & Lii, C. K. Differential effects of garlic oil and its three major organosulfur components on the hepatic detoxification system in rats. J Agric Food Chem. 2002; 50: 378-383.

25. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, DC; 1985.

26. Wang, S. T., Chen, H. W., Sheen, L. Y. & Lii, C. K. Methionine and cysteine affect glutathione level, glutathione-related enzyme activities and the expression of glutathione S-transferase isozymes in rat hepatocytes. J Nutr. 1997; 127: 2135-2141.

27. Chen, H. W., Yang, J. J., Tsai, C. W., Wu, J. J., Sheen, L. Y., Ou, C. C. & Lii, C. K. Dietary fat and garlic oil independently regulate hepatic cytochrome p(450) 2B1 and the placental form of glutathione S-transferase expression in rats. J Nutr. 2001; 131: 1438-1443.

28. Suguoka, Y., Kano, T., Okuda, A., Sakai, M., Kitagawa, T. & Muramatsu, M. Cloning and the nucleotide sequence of rat glutathione S-transferase P cDNA. Nucleic Acids Res. 1985; 13: 6049-6057.

29. Okuda, A., Sakai, M. & Muramatsu, M. The structure of the rat glutathione S-transferase P gene and related pseudogenes. J Biol Chem. 1987; 262: 3858-3863.

30. Habig, W. H., Pabst, M. J. & Jakoby, W. B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 1974; 249: 7130-7139.

31. Mannervik, B., Alin, P., Guthenberg, C., Jensson, H., Tahir, M. K., Warholm, M. & Jornvall, H. Identification of three classes of cytosolic glutathione transferase common to several mammalian species: correlation between structural data and enzymatic properties. Proc Natl Acad Sci U S A. 1985; 82: 7202-7206.

32. Tsai, C. W., Chen, H. W., Yang, J. J., Liu, K. L. & Lii, C. K. Sulfur amino acid restriction induces the π class of glutathione S-transferase expression in primary rat hepatocytes.

J Nutr. 2005; 135: 1034-1039.

33. Reddy, B. S., Rao, C. V., Rivenson, A. & Kelloff, G. Chemoprevention of colon carcinogenesis by organosulfur compounds. Cancer Res. 1993; 53: 3493-3498.

34. Henderson, C. J., Smith, A. G., Ure, J., Brown, K., Bacon, E. J. & Wolf, C. R. Increased skin tumorigenesis in mice lacking pi class glutathione S-transferases. Proc Natl Acad Sci U S A. 1998; 95: 5275-5280.

35. Munday, R. & Munday, C. M. Relative activities of organosulfur compounds derived from onions and garlic in increasing tissue activities of quinone reductase and glutathione transferase in rat tissues. Nutr Cancer. 2001; 40: 205-210.

36. Munday, R. & Munday, C. M. Low doses of diallyl disulfide, a compound derived from garlic, increase tissue activities of quinone reductase and glutathione transferase in the gastrointestinal tract of the rat. Nutr Cancer. 1999; 34: 42-48.

37. Wan, S. B., Chen, D., Dou, Q. P. & Chan, T. H. Study of the green tea polyphenols catechin-3-gallate (CG) and epicatechin-3-gallate (ECG) as proteasome inhibitors. Bioorg Med Chem. 2004; 12: 3521-3527.

38. Grune, T., Klotz, L. O., Gieche, J., Rudeck, M. & Sies, H. Protein oxidation and proteolysis by the nonradical oxidants singlet oxygen or peroxynitrite. Free Radic Biol Med. 2001; 30: 1243-1253.

39. Tuma, P. L., Nyasae, L. K. & Hubbard, A. L. Nonpolarized cells selectively sort apical proteins from cell surface to a novel compartment, but lack apical retention mechanisms. Mol Biol Cell. 2002; 13: 3400-3415.

40. Weinstein, I. B., Orenstein, J. M., Gebert, R., Kaighn, M. E. & Stadler, U. C. Growth and structural properties of epithelial cell cultures established from normal rat liver and chemically induced hepatomas. Cancer Res. 1975; 35: 253-263.

41. Tjalkens, R. B., Luckey, S. W., Kroll, D. J. & Petersen, D. R. Alpha,beta-unsaturated aldehydes increase glutathione S-transferase mRNA and protein: correlation with activation of the antioxidant response element. Arch Biochem Biophys. 1998; 359: 42-50.

42. Leung, Y. K., Ng, T. B. & Ho, J. W. Transcriptional regulation of fosl-1 by licorice in rat Clone 9 cells. Life Sci. 2003; 73: 3109-3121.

43. Diccianni, M. B., Imagawa, M. & Muramatsu, M. The dyad palindromic glutathione transferase P enhancer binds multiple factors including AP1. Nucleic Acids Res. 1992; 20: 5153-5158.

44. Xiao, D., Choi, S., Johnson, D. E., Vogel, V. G., Johnson, C. S., Trump, D. L., Lee, Y. J. & Singh, S. V. Diallyl trisulfide-induced apoptosis in human prostate cancer cells involves c-Jun N-terminal kinase and extracellular-signal regulated kinase-mediated phosphorylation of Bcl-2. Oncogene. 2004; 23: 5594-5606.

45. Reddy, S. P. & Mossman, B. T. Role and regulation of activator protein-1 in toxicant-induced responses of the lung. Am. J. Physiol. Lung Cell Mol Physiol. 2002; 283: L1161-1178.

46. Wen, J., Zhang, Y., Chen, X., Shen, L., Li, G. C. & Xu, M. Enhancement of diallyl disulfide-induced apoptosis by inhibitors of MAPKs in human HepG2 hepatoma cells. Biochem Pharmacol. 2004; 68: 323-331.

47. Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K. et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res

Commun. 1997; 236: 313-322.

48. Chen, C., Pung, D., Leong, V., Hebbar, V., Shen, G., Nair, S., Li, W., Kong, A. N. Induction of detoxifying enzymes by garlic organosulfur compounds through transcription factor Nrf2: effect of chemical structure and stress signals. Free Radic Biol Med. 2004; 37: 1578-90.

49. Morimitsu, Y., Nakagawa, Y., Hayashi, K., Fujii, H., Kumagai, T., Nakamura, Y., Osawa, T., Horio, F., Itoh, K. et al. A sulforaphane analogue that potently activates the Nrf2-dependent detoxification pathway. J Biol Chem. 2002; 277: 3456-3463.

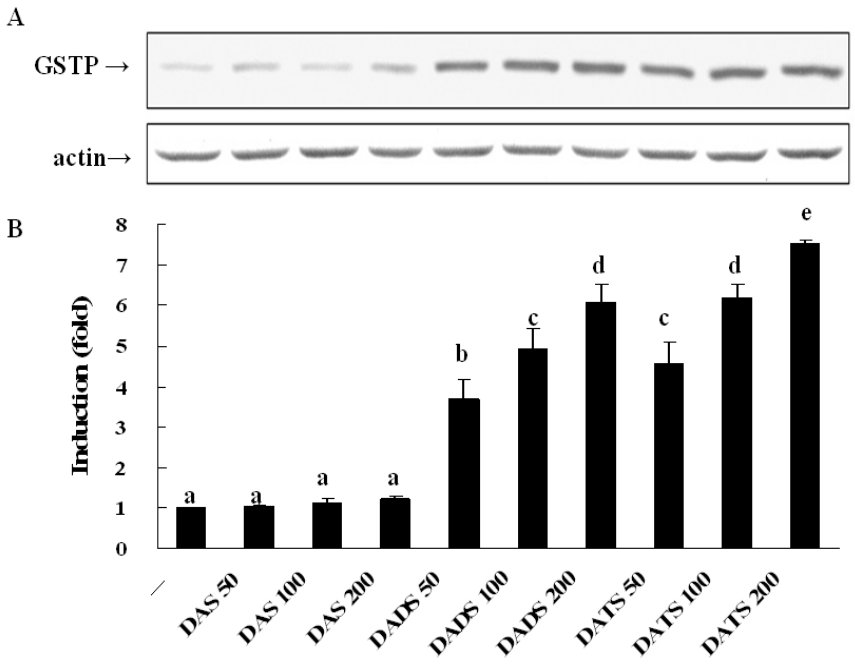


FIGURE 1 Allyl sulfide–induced protein expression of the π class of glutathione *S*-transferase (GSTP) in primary rat hepatocytes. (A) After 48 h of plating, cells were cultured with 0.1% dimethylsulfoxide alone (—) or with 50, 100, or 200 μmol/L of diallyl sulfide (DAS), diallyl disulfide (DADS), or diallyl trisulfide (DATS) for an additional 24 h. (B) Changes in GSTP protein expression were measured by densitometry. The level in control cells was regarded as 1. Each value represents the means±SD of four independent experiments. Groups not sharing a common letter differ significantly, $P<0.05$.

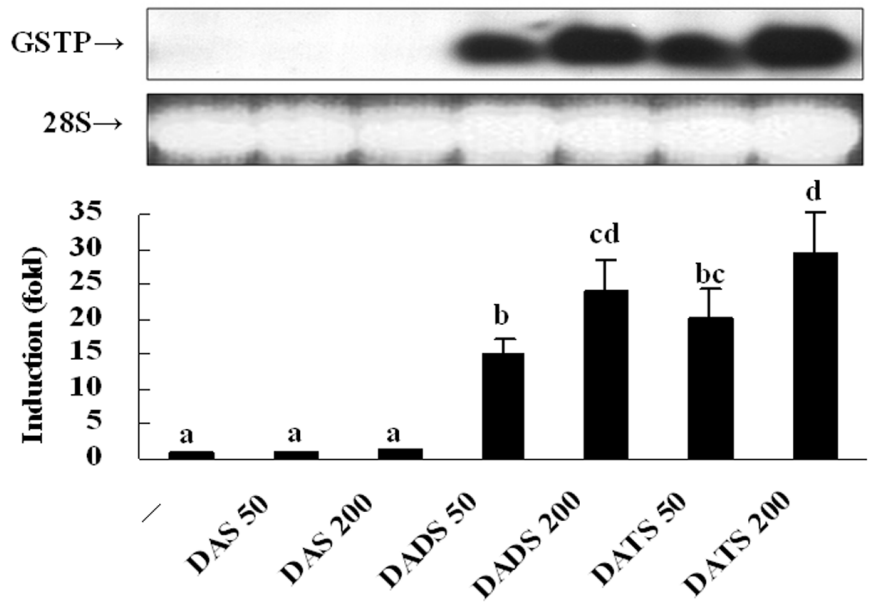


FIGURE 2 Induction of the π class of glutathione *S*-transferase (GSTP) mRNA expression by diallyl sulfide (DAS), diallyl disulfide (DADS), or diallyl trisulfide (DATS) treatment in primary rat hepatocytes. Forty-eight hours after plating, cells were treated with dimethylsulfoxide alone (—) or with 50 or 200 μmol/L of each of the garlic allyl sulfides for 24 h. The mRNA level in the control cells was regarded as 1. Values are means±SD, n=3. Groups not sharing a common letter differ significantly, P<0.05.

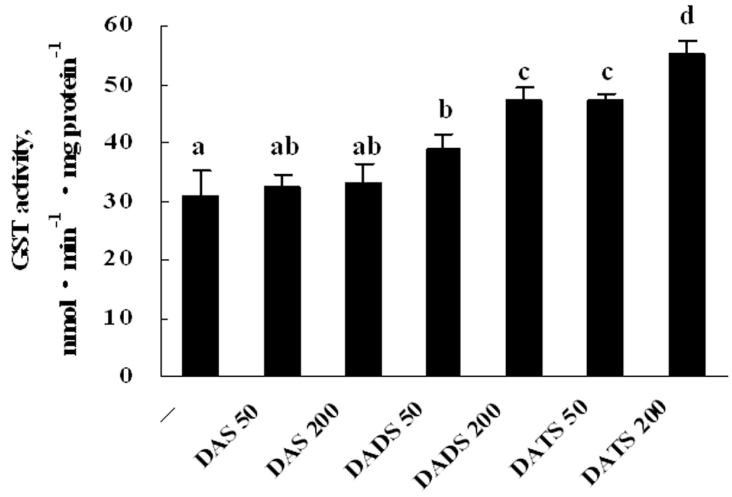


FIGURE 3 The effect of diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) on the enzyme activity of the π class of glutathione *S*-transferase (GSTP). Primary hepatocytes were treated with 0.1% dimethylsulfoxide alone (—) or with 50 or 200 μmol/L of DAS, DADS, or DATS for 24 h. Values are means±SD, n=3. Groups not sharing a common letter differ significantly, P<0.05.

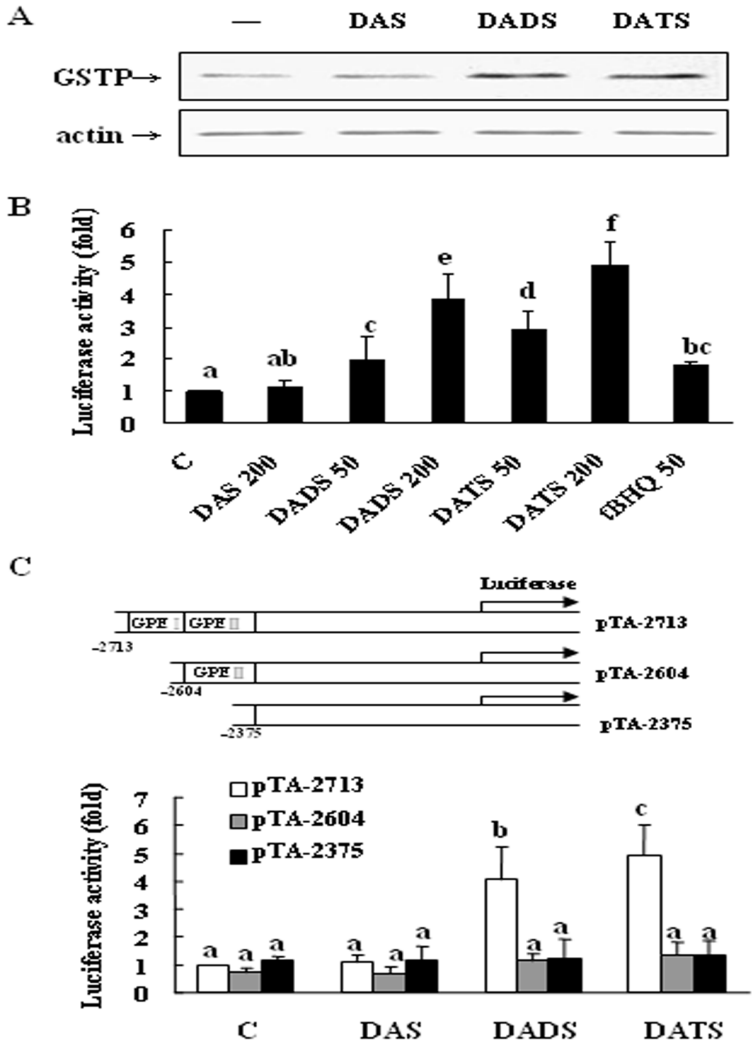


FIGURE 4 GSTP enhancer I (GPEI) is required for the up-regulation of the π class of glutathione *S*-transferase (GSTP) by garlic allyl sulfides. (A) Protein levels of GSTP in Clone 9 cells. Cells were treated with 200 μmol/L of diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) for 24 h. (B) Clone 9 cells were transfected with the pTA-GSTP Luc DNA construct (pTA-2713) and then treated with various concentrations of each of the garlic allyl sulfides or tert-butylhydroquinone (t-BHQ) for 12 h. (C) Serial deletions of the pTA-GSTP Luc DNA (pTA-2713, pTA-2604, and pTA-2375) were transfected into Clone 9 cells and then treated with 200 μmol/L of DAS, DADS, or DATS for 12 h. The activity of cells transfected with pTA-2713 and treated with dimethylsulfoxide alone (—) was regarded as 1. Values are means±SD, n=5. Groups not sharing a common letter differ significantly, $P<0.05$.

Part II:

摘要

我們進一步針對MAPK 訊號傳遞途徑,探討DADS 和 DATS 如何調節 GPE I,誘發 GSTP 表現；Clone 9 細胞分別處理 50-200 μM DADS 或 DATS，取樣進行下列分析，Electrophoretic mobility shift assay (EMSA)顯示，DADS 及 DATS 均可增加 AP-1 活化，SB600125 (JNK 抑制劑)和 PD98059 (ERK 抑制劑)則可抑制 AP-1 活化；MAPK 訊號途徑中，JNK 和 ERK 磷酸化因 DADS 和 DATS 處理呈現劑量增加，而預處理 SP600125 或 PD98059 則抑制磷酸化的發生；相較JNK 和 ERK，p38 磷酸化並不因 DADS 和 DATS 處理而變化。以上結果可知,DADS 和 DATS 這兩種脂溶性大蒜硫成分上調 GSTP mRNA 和蛋白質表現，可能係藉由 MAPK 訊號途徑中 JNK 和 ERK 活化 AP-1，進而與 GPE I 的 AP-1 結合區結合所致。

關鍵字:Clone 9 細胞、大蒜有機硫成分、π屬穀胱甘　硫轉移酶、MAPK kinases

緣由與目的

大蒜(allium sativium L., garlic)在世界各地均廣被食用，除了被視為一種食材外，它更被視為一種擁有多樣生理活性的健康食品，許多證據皆顯示大蒜的生理活性與其有機硫成分(organosulfur compounds)有關，目前為止，已被證實的包括有降血脂(Orekhov et al., 1997；Lau et al., 1987)、抗氧化(Ide et al., 1999; Lau, 2001; Wu et al., 2001)、抗血小板凝集功能(Bordia et al., 1998)以及抗腫瘤活性(Fleischauer and Arab, 2001; Velta et al., 1988)等，所以大蒜含硫成分在醫療保健方面的特殊功效一直受到許多學者的注意。在大蒜眾多生理活性中，大蒜在調節解毒能力上的作用最引起我們的興趣,先前動物實驗中，結果顯示 diallyl sulfide (DAS)、diallyl disulfide (DADS)、diallyl trisulfide (DATS)三種脂溶性成分均可增加 GSTP 酵素活性、蛋白質及mRNA 表現(Wu et al., 2001)，且其誘發程度與硫元素數目有關(DAS<DADS<DATS)，此結果令我們懷疑大蒜精油有機含硫成分的分子結構對於調控解毒酵素的表現是有差異的。目前為止，有關大蒜精油有機含硫成分如何調控 GSTP 表現的相關研究能仍很少見，所以若能結合分子生物技術的方式，便能進一步瞭解大蒜精油有機含硫成分誘發GSTP 表現之機轉，並進一步分析參與其中的相關調控因子。

材料與方法

1. clone 9 細胞的培養與處理

大鼠 clone 9 肝細胞培養於含 10% FBS 的 RPMI 1640 培養液中，分別處理 200 μM 的 DADS 和 DATS，24 小時後收取細胞，至於 JNK 抑制劑(SP600125)及 ERK 抑制劑(PD98059)處理組，則是在加入大蒜有機硫成分前預處理 1 小時，細胞液以 15000g 於 4□ 離心 30 分鐘，取細胞質液，分析 GSTP 蛋白質表現。

而在 MAPK 蛋白質(ERK, JNK, and p38)活性測定，則是在 50、100、200 μM DADS 或 DATS 處理 1 小時後，取樣分析。

2. Electrophoretic Sobility Shift Assay (EMSA)

細胞加入 200 μM DADS 或 DATS 處理 3 小時，以 cold-PBS 清洗兩次，加入 400 μl PBS，刮取細胞，2000 g 離心 5 分鐘，除去上清液，加入 200 μl hypotonic buffer (10 mM HEPES、10 mM KCl、1.0 mM $MgCl_2$、1.0 mM EDTA、0.5 mM DTT、0.2 mM PMSF、4 ug/ml leupeptin、20 μg/ml apotinin、0.5% NP-40)，使細胞懸浮，冰浴 15 分鐘，6000 g 離心 15 分鐘，吸除上層液，加入 50 μl hypertonic buffer (10 mM HEPES、400 mM KCl、1.0 mM $MgCl_2$、1.0 mM EDTA、0.5 mM DTT、0.2 mM PMSF、4 μg/ml leupeptin、20 μg/ml apotinin、10% glycerol)，4℃下振盪 30 分鐘，隨後以 10000 g 離心 15 分鐘，所得之上清液即細胞核萃出液(cell nuclear protein extract) (Shapiro et al., 1988)。取 2 μg 細胞核蛋白質與含生物素標定之 oligonucleotides 的反應液(含 50 ng/l poly (dIdC)、1X binding buffer、2.5% glycerol、5 mM $MgCl_2$ 及 0.05% NP-40)混勻，室溫反應 30 分鐘，置於 6% polyacrylamide gels 樣品槽中，電泳後，以 100 伏特於冰水浴中進行 60 分鐘轉印，將 protein-DNA 結合物轉印至 HyBond N^+膜上，UV 燈下 cross-link 5-10 分鐘，Blocking 反應後加入 streptavidin-horseradish peroxidase，並以 ECL kit 呈色，置於感光夾中以 X 光片進行顯影反應。

結果與討論

一、 DADS 和 DATS 對轉錄因子 AP-1 活化之作用

結果如圖一所示，DADS 和 DATS 皆以劑量關係增加細胞核內轉錄因子AP-1 與 AP-1 binding probe 序列結合，且 DATS 作用大於 DADS，JNK 抑制劑(SP600125)及 ERK 抑制劑(PD98059)預處理均可抑制 AP-1 活化，Cold AP-1 binding probe 抑制 AP-1 與 hot AP-1 binding probe 序列的結合，顯示圖中所示為 AP-1 專一性結合；除 DADS 和 DATS 外，本實驗亦使用一已知透過 AP-1 活化誘發多種 phase II 解毒酵素的抗氧化分子- t-butyl hydroquinone (tBHQ)作為正對照組，結果顯示 50μM tBHQ 確實增加此結合能力，但誘發效果低於 DATS。

二、 DADS、DATS 與 MAPK kinases 蛋白質表現

如圖二所示，DADS 和 DATS 皆以劑量關係增加 JNK 和 ERK 的磷酸化，而預處理 JNK 抑制劑(SP600125)或 ERK 抑制劑(PD98059)則抑制磷酸化的發生。相較於 JNK 和 ERK，另一 MAPK kinase-p38 的磷酸化，並不因 DADS 和 DATS 的處理而增加。我們亦同時觀察 JNK 及 ERK 抑制劑是否影響 DADS 和 DATS 誘發 GSTP 蛋白質表現，結果由圖三，SP600125 和 PD98059 確實會降低 GSTP 蛋白質表現。

以上結果可知，DADS 和 DATS 誘發 GSTP 酵素表現極可能與 JNK 及 ERK 訊號途徑的活化有關。

參考文獻

Bordia, A., Verma, S.K. and Srivastava, K.C. (1998) Effects of garlic (Allium sativum) on blood lipid, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease. Prostaglandins Leuko. Essent. Fatty Acids 58: 257-263.

Fleischhauer A.T. and Arab L. (2001) Garlic and cancer: a critical review of the epidemiologic literature. J. Nutr. 131: 1032S-1040S.

Ide, N and Lau, B.H.S. (1999) S-allylcysteine attenuates oxidative stress in endothelial cells. Drug Dev. Ind. Pharm. Acol. 25: 619-24.

Lau, B.H.S., Yamasaki, T. and Gridley, D.S. (1991) Garlic compounds modulate macrophage and T-lymphocyte functions. Mol. Biother. 3: 103-107.

Lau, B., Lam, F. and Wang-Cheng, R. (1987) Effect of garlic preparation on blood lipid. Nutr. Res. 7: 139-141.

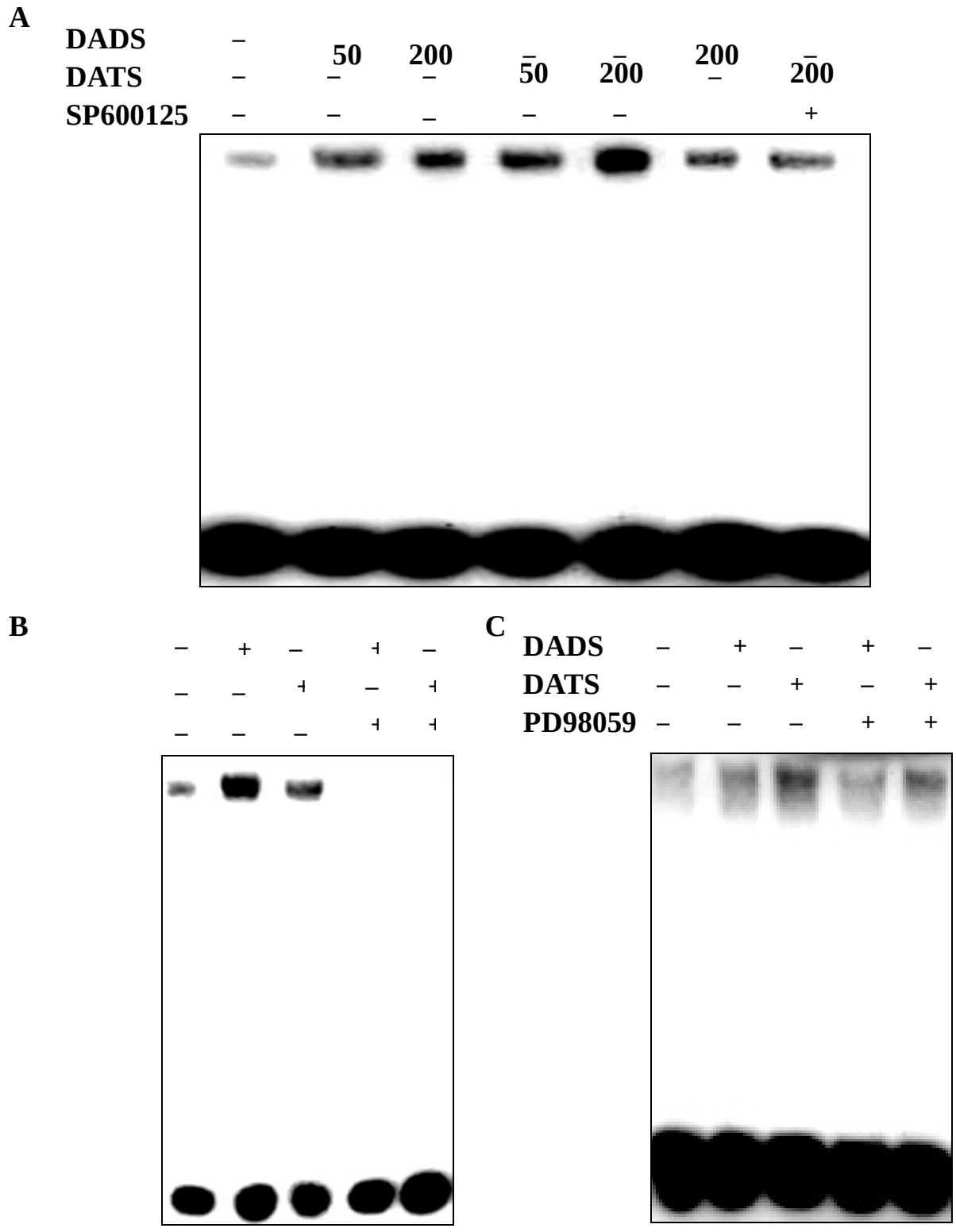
Orekhov, A.N. and Tertov, V.V. (1997) In vitro effect of garlic extract on lipid content in normal and atherosclerotic human aortic cells. Lipids 32: 1055-1060.

Velta, L.S. , George, B. and Lee, W.W. (1988) Effect of organosulfur compounds from garlic and onion on benzo(a)pyrene-induced neplasia and glutathione S-transferase activity in the mouse. Carcinogenesis 9 : 131-4.

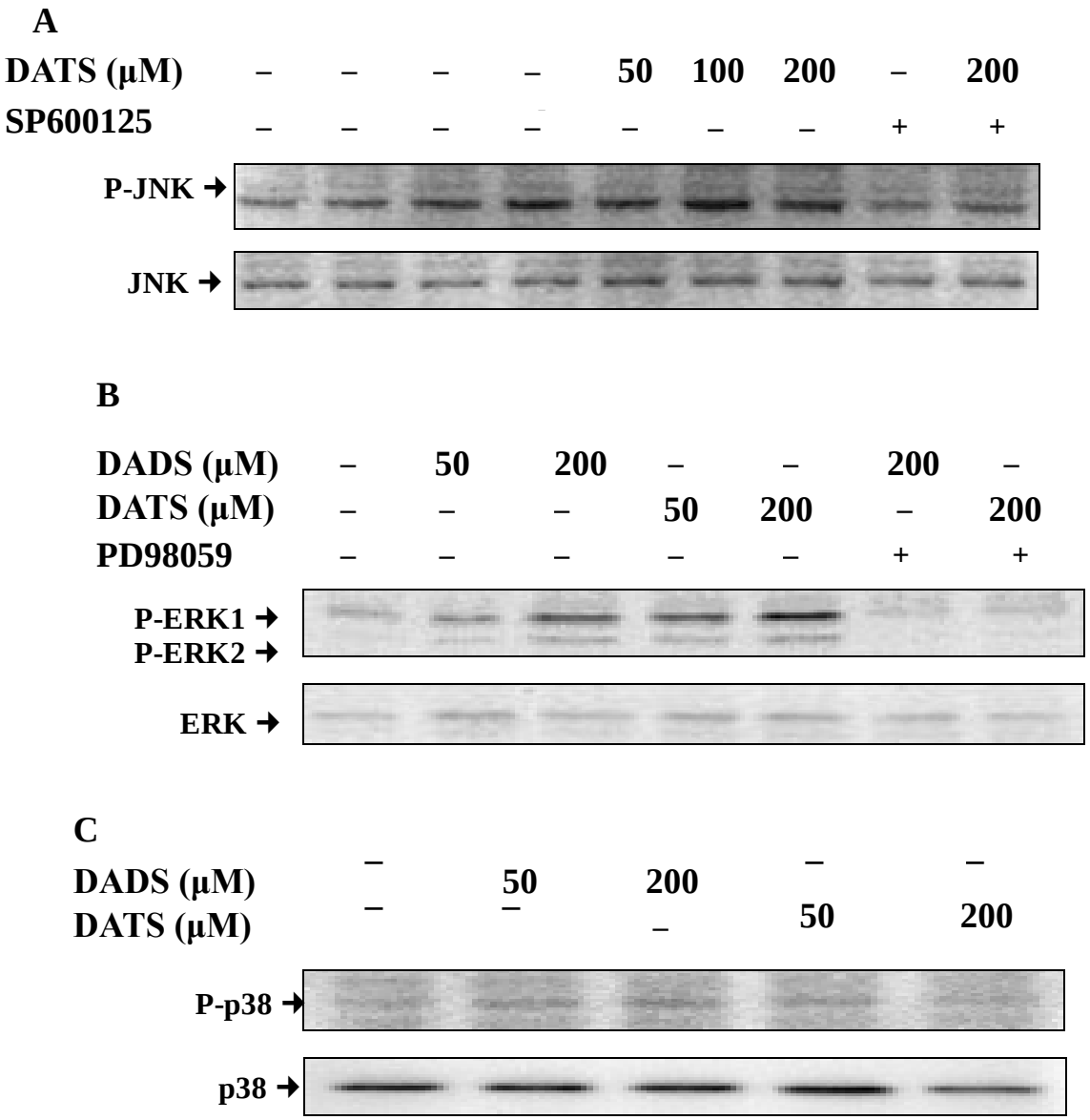
Wu, C.-C., Sheen, L. Y., Chen, H.-W., Tsai, S.-J. and Lii, C.-K. (2001) Effects of organosulfur compounds from garlic oil on the antioxidation system in rat liver and red blood cells. Food Chem. Toxicol. 39: 563-569.

圖表

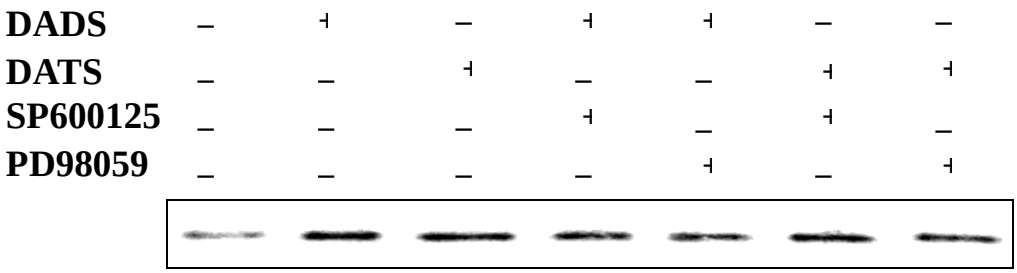
圖一、DADS 和 DATS 對細胞核內轉錄因子 AP-1 與 AP-1 binding probe 序列結合之作用



圖二、DADS 和 DATS 對 MAPK kinase 蛋白質表現的影響



圖三、SP600125 與 PD98059 對 GSTP 蛋白質表現的影響



總結論

DADS和DATS藉由MAPK訊號途徑中JNK和ERK活化AP-1，進而與GSTP啟動區-2.7至-2.6 kb間GPEⅠ中的AP-1結合區結合，因而上調(up-regulate)GSTP mRNA和蛋白質的表現。