Phytomedicine xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells *in vitro* and *in vivo*

Kuang-Chi Lai[a,b], Chao-Lin Kuo[c], Heng-Chien Ho[d], Jai-Sing Yang[e], Chia-Yu Ma[f], Hsu-Feng Lu[g,h], Hui-Ying Huang[i], Fu-Shin Chueh[j], Chien-Chih Yu[k,*,1], Jing-Gung Chung[l,m,**,1]

[a] *Department of Surgery, China Medical University Beigang Hospital, Yunlin 651, Taiwan*
[b] *School of Medicine, China Medical University, Taichung 404, Taiwan*
[c] *School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, China Medical University, Taichung 404, Taiwan*
[d] *Department of Biochemistry, China Medical University, Taichung 404, Taiwan*
[e] *Department of Pharmacology, China Medical University, Taichung 404, Taiwan*
[f] *Department of Food and Beverage Management, Taipei Chengshih University of Science and Technology, Taipei 112, Taiwan*
[g] *Department of Clinical Pathology, Cheng Hsin General Hospital, Taipei 112, Taiwan*
[h] *College of Human Ecology, Fu-Jen Catholic University, New Taipei 242, Taiwan*
[i] *Department of Nutrition, China Medical University, Taichung 404, Taiwan*
[j] *Department of Health and Nutrition Biotechnology, Asia University, Taichung 413, Taiwan*
[k] *School of Pharmacy, China Medical University, Taichung 404, Taiwan*
[l] *Department of Biological Science and Technology, China Medical University, Taichung 404, Taiwan*
[m] *Department of Biotechnology, Asia University, Taichung 413, Taiwan*

ARTICLE INFO

Keywords:
Garlic
Diallyl polysulfides
Drug resistant gene
Colo 205 human colon cancer cells

ABSTRACT

To elevate chemo-resistance of human cancer cells is a major obstacle in the treatment and management of malignant cancers. Diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS) are presented in the *Alliaceae* family particularly in garlic. Although DAS, DADS and DATS have been shown to exhibit anticancer activities, there is little information on effects of these compounds on drug resistant genes in human colon cancer cells *in vitro* and *in vivo*. Herein, we are the first to show that DAS, DADS and DATS at 25 μM for 24-h and 48-h incubations promoted expression of drug resistant genes in colo 205 human colon cancer cells. *In vitro* experiments indicated that DATS promoted gene expression of *multidrug resistant 1* (*Mdr1*) ($p < 0.05$), and DAS and DADS promoted *MRP3* gene expression and DATS alone stimulated gene expression of *multidrug resistance-associated protein-1* (*MRP1*) ($p < 0.05$) in colo 205 cells. *In vivo* studies demonstrated that DADS and DATS induced *Mdr1* and *MRP1* gene expression ($p < 0.05$). DADS promoted *MRP3* gene expression ($p < 0.05$) as well as DADS and DATS increased *MRP4* and *MRP6* gene expression ($p < 0.05$) in the colo 205 xenograft mice. Based on our *in vitro* and *in vivo* results, diallyl polysulfides (DAS, DADS and DATS) affected the gene expression of the multidrug resistance in colo 205 human colon cancer cells *in vitro* and *in vivo*.

Introduction

Drug resistance is a major impediment in the treatment of cancer (Harnett et al. 1987; Liem et al. 2002). Multiple cytotoxic drugs with diverse mechanisms of action have not been effective as cancer cells developed resistance simultaneously to different anticancer drugs. Advances in understanding the mechanisms of multidrug resistance (MDR) and associated multidrug-resistant genes in tumor cells or tissues models are essential to improve cancer therapy (Chao et al. 1991; Fan et al. 2004; Perez-Tomas 2006). It is reported that MDR of mammalian cancer cells exhibited that overexpression of permeability-glycoproteins (P-gps) including a family of mdr genes in membrane and alterations of ATP-dependent drug efflux and intracellular drug accumulation (Callen et al. 1987; Fan et al. 2004). Therefore, the novel investigations for exploring their functions of cancer drug-resistance mechanism are vital during cancer treatment.

Diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS) are the sulfur-containing compounds found in the *Alliaceae* family such as garlic. These three compounds are thought to be only part responsible for health-promoting effects such as

* Corresponding author at: School of Pharmacy, China Medical University, No. 91, Hsueh-Shih Road, Taichung 40402, Taiwan. Tel.: +886 422053366x5113.
** Corresponding author at: Department of Biological Science and Technology, China Medical University, No. 91, Hsueh-Shih Road, Taichung 40402, Taiwan. Tel.: +886 422053366x2161; fax: +886 422053764.
E-mail addresses: ccyu@mail.cmu.edu.tw (C.-C. Yu), jgchung@mail.cmu.edu.tw (J.-G. Chung).
[1] Both authors contributed equally to this work.

0944-7113/$ – see front matter © 2012 Published by Elsevier GmbH.
doi:10.1016/j.phymed.2012.02.004

antimicrobial, hypolipidemic, antithrombotic, and antitumor activities (Augusti 1996; Block et al. 2001; Milner 2001; Yin and Tsao 1999). Epidemiologic studies and laboratory experiments have demonstrated that garlic has bioactivity and anticancer effects (Bianchini and Vainio 2001; Fleischauer and Arab 2001; Singh and Shukla 1998). It is reported that DAS, DADS and DATS are agonist of both transient receptor potential cation channel, subfamily A, member 1 (TRPA1) and transient receptor potential cation channel, subfamily V, member 1 (TRPV1) but with high affinity for TRPA1 activity (Koizumi et al. 2009).

DAS enhanced antioxidants and suppresses inflammatory cytokines through the activation of Nrf2 and it was protective against oxidative stress induced by gentamicin in Wistar rats (Kalayarasan et al. 2009). DAS reduced INH-induced toxicity by stabilizing the cellular GSH content from oxidative injury in rat liver cells (Zhai et al. 2008). DAS exerted a protective role on liver function and tissue integrity in the face of enhanced tumorigenesis caused by N-nitrosodiethylamine, as well as improving cancer-cell sensitivity to chemotherapy (Ibrahim and Nassar 2008).

DADS inhibited the proliferation of various types of human cancer cells such as breast (Nakagawa et al. 2001), lung (Hong et al. 2000), leukemia (Kwon et al. 2002), neuroblastoma (Filomeni et al. 2003) and colon (Bottone et al. 2002; Park et al. 2002; Sundaram and Milner 1996) cancer cells through induction of cell cycle arrest or apoptosis. DADS exhibited antioxidant properties as it increases the intracellular content of reduced glutathione (Bose et al. 2002; Wu et al. 2001). Recently, we reported that DADS induced apoptosis in human colon cancer cells through ROS, caspases- and mitochondria-dependent pathways (Yang et al. 2009).

DATS-induced G2/M phase cell cycle arrest was associated with reactive oxygen species (ROS)-dependent hyperphosphorylation and destruction of the cell division cycle 25C (Cdc25C) phosphatase in prostate cancer cells (Xiao et al. 2005). This effect was selective for cancer cells since a normal prostate epithelial cell line was resistant to cell cycle arrest by DATS (Xiao et al. 2005). DATS increases labile iron levels due to c-Jun N-terminal kinase (JNK)-mediated degradation of the iron storage protein ferritin in prostate cancer cells (Antosiewicz et al. 2006). DATS induced apoptosis in PC-3 and DU145 human prostate cancer cells *via* inhibiting the expression of Bcl-2 protein, and activating ERK1/2 and JNK pathways but inactivating the Akt signaling axis (Xiao et al. 2004; Xiao and Singh 2006).

DAS, DADS and DATS can induce cell cycle arrest and apoptosis in many types of human cancer cell lines, but there is no information to address DAS, DADS and DATS-affected drug resistance genes either *in vitro* or *in vivo* studies. In the present study, we investigated the effects of DAS, DADS and DATS on drug resistant gene expression in colo 205 cells. DAS, DADS and DATS also stimulated specific gene associated with multi-drug resistance in colo 205 human colon cancer cells.

Materials and methods

Chemicals and reagents

DAS, DADS, dimethyl sulfoxide (DMSO) and trypan blue were obtained from Sigma–Aldrich Corp. (St. Louis, MO, USA). DATS was purchased from LKT Laboratories, Inc. (St. Paul, MN, USA). RPMI-1640 medium, fetal bovine serum, L-glutamine, penicillin–streptomycin and trypsin-EDTA were obtained from Invitrogen/Life Technologies (Carlsbad, CA, USA).

Cell culture

The human colon cancer cell line (colo 205) was obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). The cells were maintained in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin–streptomycin (100 units/ml penicillin and 100 μg/ml streptomycin) and 2 mM L-glutamine in 75-cm^2 tissue culture flasks and grown at 37 °C under a humidified 5% CO_2 and 95% air at one atmosphere.

Table 1
The DNA sequence was evaluated using the primer express software.

Primer name	Primer sequence
homo Mdr1-F	GTGTGGTGAGTCAGGAACCTGTAT
homo Mdr1-R	TCTCAATCTCATCCATGGTGACA
homo MRP1-F	CCTCAGCATCTTCCTTTTCATGT
homo MRP1-R	TCCTGAGTCCCGTTGACGAT
homo MRP3-F	GGAGTCGCTTTCATGGTCTTG
homo MRP3-R	TGATGCGCGAGTCCTTCA
homo MRP4-F	CTTCTTCCCCTCAGCCATTG
homo MRP4-R	ACGGTTGCGCTGTGATATCTC
homo MRP6-F	TTAGACGCGAGAGGTCCATCA
homo MRP6-R	CGTATTGGATGCTGTCCTTTCC
homo GAPDH-F	ACACCCACTCCTCCACCTTT
homo GAPDH-R	TAGCCAAATTCGTTGTCATACC

Mdr, multi-drug resistance gene; MRP, multidrug resistance protein. Relative quantification was done by using GAPDH as endogenous control.

In vitro studies

Real-time polymerase chain reaction (PCR)

It was used to examine effects of DAS, DADS and DATS on multidrug resistance genes in colo 205 cells. Cells (2×10^6 cells/well) in RPMI 1640 medium were plated in 12-well plates and allowed to grow for 24 h. The medium was replaced with fresh complete medium containing 25 μM of DAS, DADS and DATS, respectively, for 24 and 48 h based on our earlier studies (Lai et al. 2011; Yang et al. 2009). Stock solutions of DAS, DADS and DATS were dissolved in DMSO, and an equal volume of DMSO (final concentration 0.1%) was added to cells as a vehicle control. At the end of the incubation period, cells were collected and suspended in PBS by centrifugation. Total RNA was isolated using the Qiagen RNeasy Mini Kit (Qiagen, Inc., Valencia, CA, USA) as previously described (Chiang et al. 2011; Ji et al. 2009; Lu et al. 2010). RNA samples were reverse-transcribed for 30 min at 42 °C with High Capacity cDNA Reverse Transcription Kit according to the standard protocol of the supplier (Applied Biosystems/Life Technologies, Carlsbad, CA, USA). Quantitative PCR conditions were: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C, 1 min at 60 °C using 1 μl of the cDNA reverse-transcribed as described above, 2X SYBR Green PCR Master Mix (Applied Biosystems) and 200 nM of forward (F) and reverse (R) primers as shown in Table 1. Each assay was run on an Applied Biosystems 7300 Real-Time PCR system (Applied Biosystems/Life Technologies) in triplicate and expression fold-changes were derived using the comparative C_T method (Chiang et al. 2011; Lu et al. 2010).

In vivo studies

Mouse xenograft model

The mice experiments were conducted according to institutional guidelines approved by the Institutional Animal Care and Use Committee (IACUC; No. 95-43-N), China Medical University (Taichung, Taiwan). Thirty six-week-old male athymic nude mice were obtained from the Laboratory Animal Center of National Applied Research Laboratories (Taipei, Taiwan). Animals were maintained in standard vinyl cages with air filter tops in a filtered laminar air flow room, food and water were autoclaved and provided *ad libitum*. The outline of the experimental design is shown in Fig. 3.

The colo 205 cells (1×10^7 per mouse) were subcutaneously (*s.c.*) injected into the flanks of mice as described previously

Please cite this article in press as: Lai, K.-C., et al., Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells *in vitro* and *in vivo*. Phytomedicine (2012), doi:10.1016/j.phymed.2012.02.004

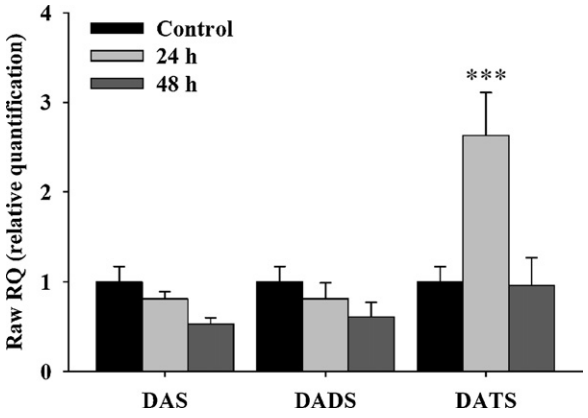

Fig. 1. DAS, DADS and DATS affected *Mdr1* gene expression in colo 205 cells. Cells were individually treated with 25 μM DAS, DADS and DATS for 24 and 48 h, and then cells were harvest for real-time PCR to determine the gene expression of *Mdr1* as described in "Materials and Methods". ***$p < 0.001$.

(Ho et al. 2009; Ji et al. 2009). Mice bearing tumors were randomly divided into treatment groups (six mice per group). When xenografts reached volumes of about 100 mm^3, the animals were then intraperitoneally (*i.p.*) injected once every 4 days (in the morning) with 30 μl of control vehicle (DMSO), DAS or DADS or DATS (6 mg/kg), and doxorubicin (8 mg/kg). Mice exhibiting tumors were monitored and counted, and the tumor sizes were measured initially after 10 days for up to 32 days after tumor cell inoculation. At the end of the study (4 weeks after cell inoculation), animals were photographed and sacrificed. Tumors were removed, measured and weighted. Total RNA from tumor tissues in each group was collected for real-time PCR to examine multi-drug resistant genes expression as described above.

Statistical analysis

Each value represents mean ± SD between the control and DAS, DADS and DATS-treated groups that were compared by one-way ANOVA followed by Dunnett's test. *$p < 0.05$ and ***$p < 0.001$ were considered significant.

Results

DAS, DADS and DATS affected Mdr1 gene expression in colo 205 cells

Real-time PCR data of *Mdr1* gene expression in colo 205 cells after treatment with DAS, DADS and DATS are shown in Fig. 1. DATS promoted *Mdr1* gene expression. However, DAS and DADS did not affect *Mdr1* gene expression in colo 205 cells.

DAS, DADS and DATS affected MRPs genes expression in colo 205 cells

Cells were treated with 25 μM DAS, DADS and DATS for 24 and 48 h and gene expression levels in colo 205 cells were determined for *MRP1, MRP3, MRP4* and *MRP6*. Results presented in Fig. 2A–C indicated that DAS and DADS enhanced *Mdr3* gene expression at the 48-h treatment, but did not affect *MRP1, MRP4* and *MRP6* gene expression in colo 205 cells (Fig. 2A and B). However, DAS and DADS inhibited the gene expressions of MRPs at 24 and 48-h treatment, and both inhibited *MRP3* at 24-h treatment and inhibited *MRP4* at 48 h treatment in colo 205 cells. Furthermore, DADS treatment for 48 h inhibited *MRP6* gene expressions. DATS treatment for 24 h led to significant inhibition of *MRP3* gene expressions in colo 205 cells.

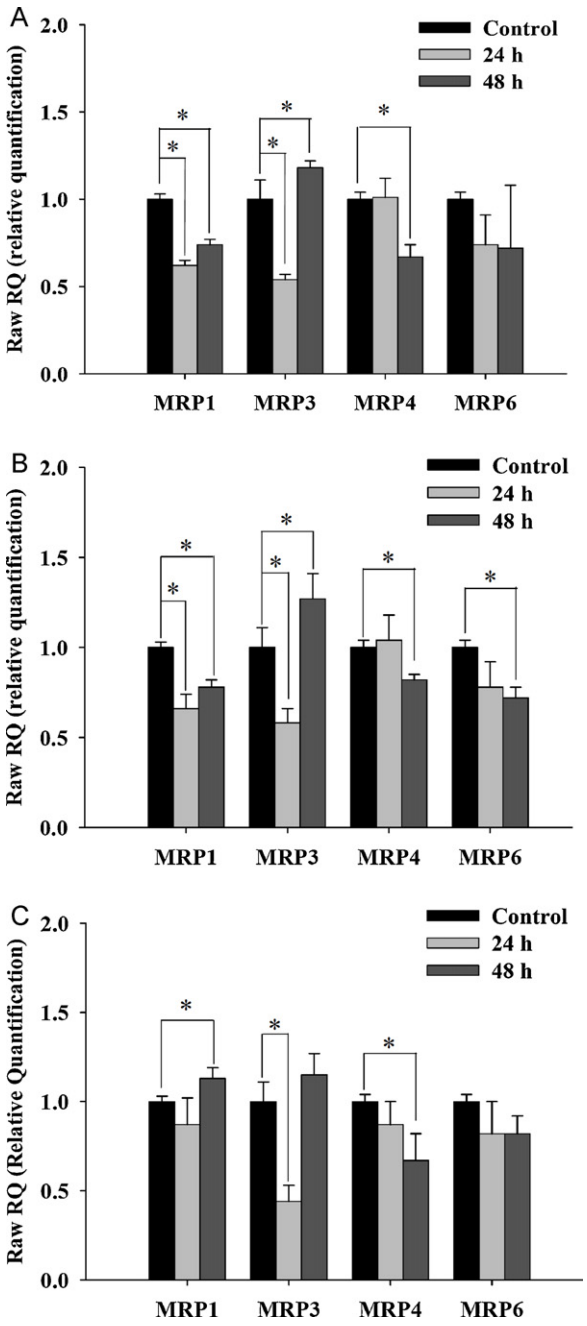

Fig. 2. DAS, DADS and DATS affected *MRPs* genes expression in colo 205 cells. Cells were individually treated with 25 μM DAS (A), DADS (B) and DATS (C) for 24 and 48 h then cells were harvest for real-time PCR to determine the gene expression of *MRP1*, *MRP3*, *MRP4* and *MRP6* as described in "Materials and Methods". *$p < 0.05$.

It can be seen in Fig. 2C that DATS promoted *MRP1* gene expression at the 48 h treatment but did not affect *MRP3*, *MRP4* and *MRP6* gene expression.

Representative tumors in the xenograft animal model

Thirty nude mice were *s.c.* implanted with 1×10^7 colo 205 cells for 10 days and then randomly divided into 5 groups for treatment with DMSO, 8 mg/kg doxorubicin, 6 mg/kg DAS, 6 mg/kg DADS and 6 mg/kg DATS. And representative animals with tumors are shown in Fig. 4A and B. Results shown in Fig. 4C and D indicated that DADS and DATS significantly suppressed xenograft tumors (weight and size) in comparisons to un-treated control group.

Please cite this article in press as: Lai, K.-C., et al., Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells *in vitro* and *in vivo*. Phytomedicine (2012), doi:10.1016/j.phymed.2012.02.004

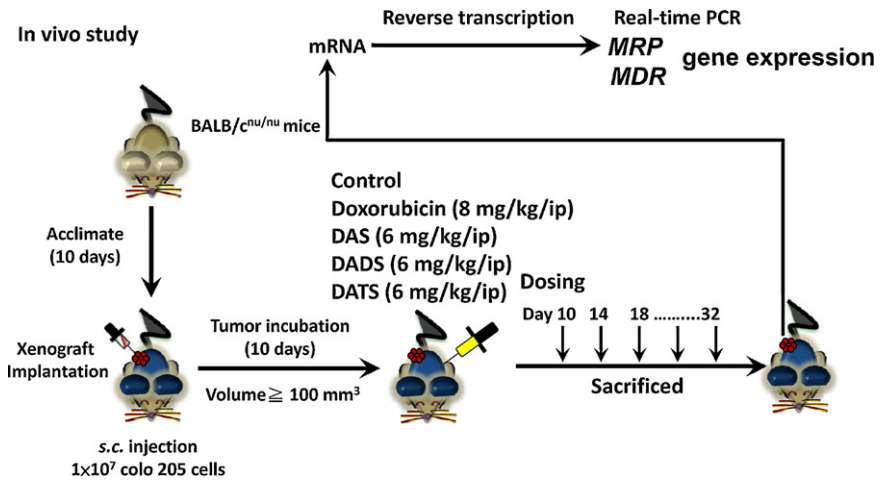

Fig. 3. Experimental design of DAS, DADS and DATS-affected gene expression in a colo 205 xenograft tumor animal model. The animal were subcutaneously implanted with colo 205 cells for 10 days until tumor around 100 mm^3 then randomly divided into 5 groups. Group 1 was treated with DMSO only. Group 2 was treated with 8 mg/kg doxorubicin. Group 3 was treated with 6 mg/kg DAS. Group 4 was treated with 6 mg/kg DADS. Group 5 was treated with 6 mg/kg DATS. All mice were treated for 34 days. During the treatment, each animal was measure tumor size and weight as described in "Materials and Methods".

DAS, DADS and DATS affected MRPs genes expression in colo 205 cells in vivo

Fig. 5A and B show that DATS > DADS on increasing gene expression of *Mdr1* and *MRP1*. DAS did not significantly alter *Mdr1* and *MRP1* gene expression levels. Data in Fig. 5C indicated that only DADS stimulated *MRP3* gene expression and showed that DADS and DATS promoted *MRP4* and *MRP6* gene expression as seen in Fig. 5D and E.

Discussion

Cancer cells are able to develop resistance simultaneously to many different anticancer drugs (Perez-Tomas 2006). Cancer cell resistance to chemotherapy involves several mechanisms including mutation, drug inactivation, over-expressions of the drug target genes or elimination of the drug from the cell. Goldstein et al. showed that MDR1 was expressed in epithelial cancers derived from various organs/tissue (colon, liver, kidney) and it was found in hematopoeitic cancers (AML, ALL lymphoma) and solid tumors (breast and ovary cancer) (Goldstein et al. 1989). The human *MDR1* gene lies on chromosome 7 at q21.1 and its polymorphisms have been reported in the *MDR1* gene since 1989 (Callen et al. 1987).

Many reports have shown that DAS, DADS and DATS inhibited proliferation and apoptosis in different human cancer cell lines, including colon cancer cell lines. However, the effects of DAS, DADS and DATS on MDR gene expression in colon cancer cells have not been fully reported. The previous report described by Demeule et al. indicated that DADS has an effect of drug resistance

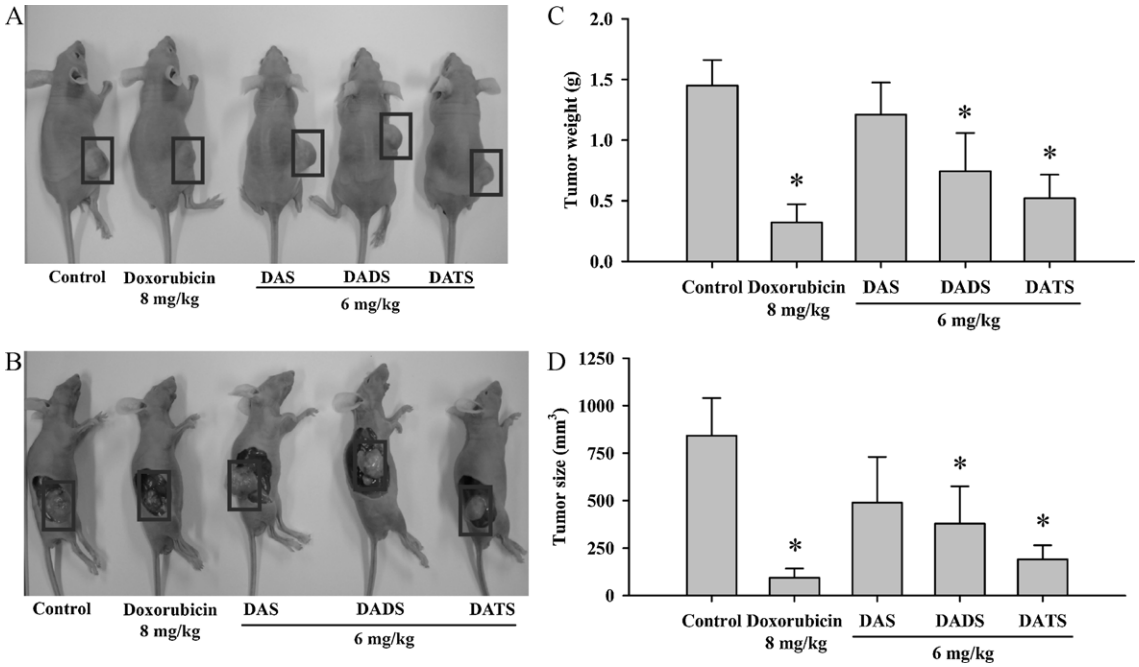

Fig. 4. Representative tumor on the xenograft animal model. Thirty nude mice were subcutaneously injected with 1×10^7 colo 205 cells/mouse for 10-days incubation and then randomly divided into 5 groups. (A) Group 1 was treated with DMSO only. Group 2 was treated with 8 mg/kg doxorubicin. Group 3 was treated with 6 mg/kg DAS. Group 4 was treated with 6 mg/kg DADS. Group 5 was treated with 6 mg/kg DATS. All were treated for 34 days. (B) Respective images were shown after all animal were sacrificed. During the treatment, each animal was measured (C) tumor weight and (D) size as described in "Materials and Methods".

Please cite this article in press as: Lai, K.-C., et al., Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells *in vitro* and *in vivo*. Phytomedicine (2012), doi:10.1016/j.phymed.2012.02.004

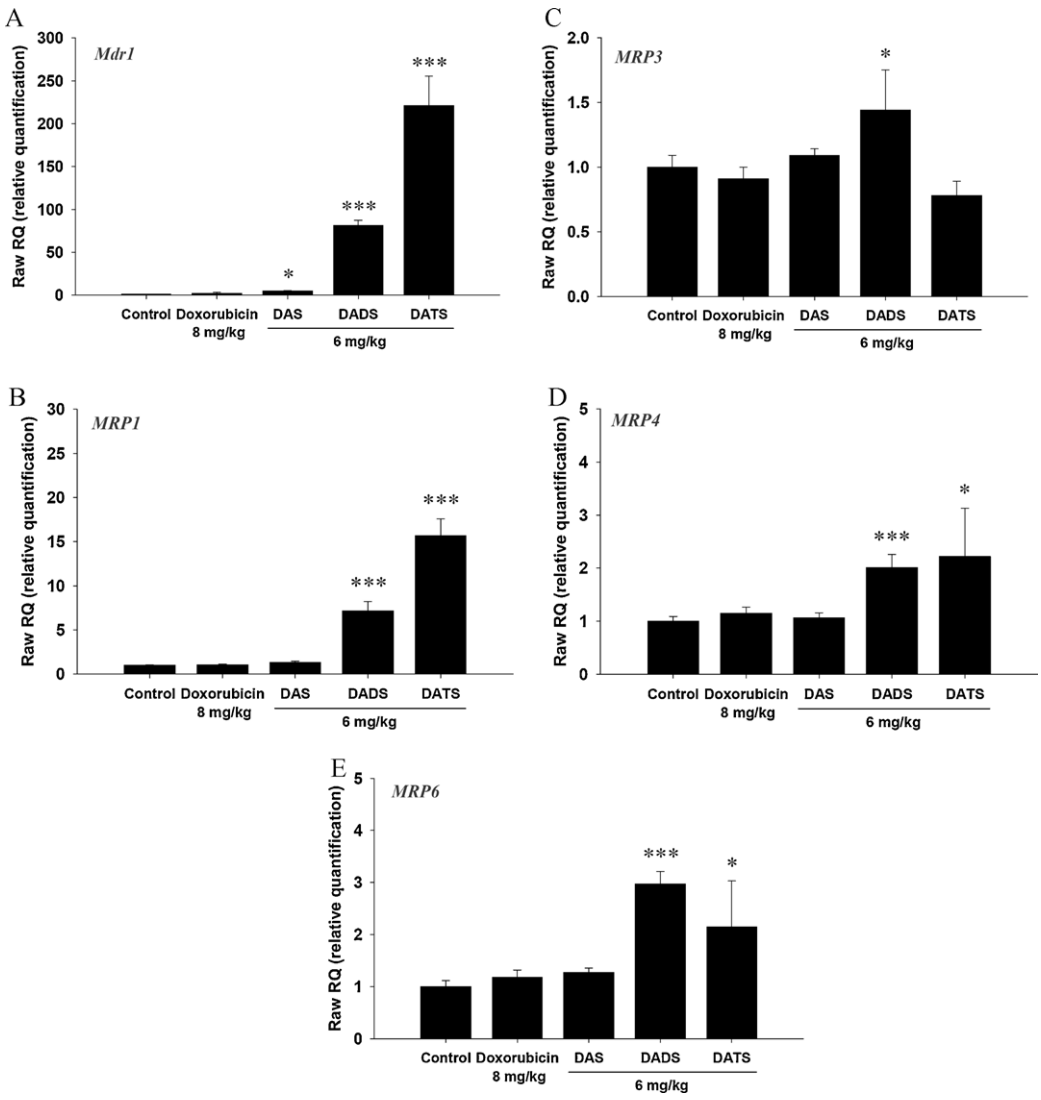

Fig. 5. DAS, DADS and DATS affected *Mdr1* and *MRPs* genes expression in colo 205 cells. Thirty nude mice were subcutaneously implanted with 1×10^7 colo 205 cells per mouse for 10 days and then randomly divided into 5 groups. Group 1 was treated with DMSO only. Group 2 was treated with 8 mg/kg doxorubicin. Group 3 was treated with 6 mg/kg DAS. Group 4 was treated with 6 mg/kg DADS. Group 5 was treated with 6 mg/kg DATS. All mice were treated for 34 days and then these animals were sacrificed. Tumors were collected for extraction of total RNA then for *Mdr1* (A), *MRP1* (B), *MRP3* (C), *MRP4* (D) and *MRP6* (E) genes expression which was observed in DMSO, doxorubicin and DAS, DADS and DATS-treated groups were compared and analyzed by one-way ANOVA followed by Dunnett's test. $^*p < 0.05$ and $^{***}p < 0.001$.

and is able to increase P-glycoprotein and promote the expression of multidrug resistance-associated protein 2 (MRP-2) *in vivo* (Demeule et al. 2004). In this study, we investigated and examined the effects of DAS, DADS and DATS on MDR gene expression in human colon cancer cells (colo 205). DATS promoted *Mdr1* gene expression in colo 205 cells, but DAS and DADS did not affect *Mdr1* gene expression (Fig. 1). In *in vivo* studies, our results also showed DADS and DATS promoted *Mdr1* gene expression but DATS in xenograft colo 205 tumors after colo 205 cells were injected into mice. Instead, DAS did not affect *Mdr1* gene expression *in vivo* (Fig. 5A).

The human *MRP1* gene is mapped to chromosome 16p13.1 (Slovak et al. 1993) and encompasses at least 200,000 base pairs containing 31 exons (Grant et al. 1997). Cells overexpressing MRP1 protein are resistant to a wide variety of anticancer drugs including doxorubicin (Chang et al. 1997), suggesting that they may be the substrates of MRP1 protein. In the *in vitro* studies, we showed that colo 205 cells after exposure to DAS, DADS and DATS for 24 and 48 h that DAS and DADS promoted *Mdr3* gene expression at the 48 h treatment but did not affect *MRP1, MRP4* and *MRP6* gene expression levels (Fig. 2A and B). DATS promoted *MRP1* gene expression at 48 h treatment but did not affect *MRP3, MRP4* and *MRP6* gene expression.

Several studies have shown that DAS, DADS and DATS induced cytotoxicity in colon cancer cells and the order of effects was DAS < DADS < DATS. However, the effects of these compounds on expression levels of MDR genes are not exactly clear. Our results showed that DAS, DADS and DATS presented different effects on drug-resistant gene expression levels in colo 205 cells *in vitro* and *in vivo*. DATS has greater stimulatory effects on drug resistance gene expression levels but cytotoxicity *in vitro* and *in vivo* is higher for DATS than that of DAS and DADS. Transcriptional activity may not be the primary mechanism for MDR induced by DATS, DAS and DADS. Apparently, further investigations are needed in the future.

Acknowledgement

This work was supported by the grant NSC 95-2320-B-039-030-MY2 from National Science Council, Republic of China (Taiwan) and by the grant CMUBH R100-009 from China Medical University Beigang Hospital, Yunlin, Taiwan R.O.C.

Please cite this article in press as: Lai, K.-C., et al., Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells *in vitro* and *in vivo*. Phytomedicine (2012), doi:10.1016/j.phymed.2012.02.004

References

Antosiewicz, J., Herman-Antosiewicz, A., Marynowski, S.W., Singh, S.V., 2006. c-Jun NH(2)-terminal kinase signaling axis regulates diallyl trisulfide-induced generation of reactive oxygen species and cell cycle arrest in human prostate cancer cells. Cancer Res. 66, 5379–5386.

Augusti, K.T., 1996. Therapeutic values of onion (Allium cepa L.) and garlic (Allium sativum L.). Indian J. Exp. Biol. 34, 634–640.

Bianchini, F., Vainio, H., 2001. Allium vegetables and organosulfur compounds: do they help prevent cancer? Environ. Health Perspect. 109, 893–902.

Block, E., Birringer, M., Jiang, W., Nakahodo, T., Thompson, H.J., Toscano, P.J., et al., 2001. Allium chemistry: synthesis, natural occurrence, biological activity, and chemistry of Se-alk(en)ylselenocysteines and their gamma-glutamyl derivatives and oxidation products. J. Agric. Food Chem. 49, 458–470.

Bose, C., Guo, J., Zimniak, L., Srivastava, S.K., Singh, S.P., Zimniak, P., et al., 2002. Critical role of allyl groups and disulfide chain in induction of Pi class glutathione transferase in mouse tissues in vivo by diallyl disulfide, a naturally occurring chemopreventive agent in garlic. Carcinogenesis 23, 1661–1665.

Bottone Jr., F.G., Baek, S.J., Nixon, J.B., Eling, T.E., 2002. Diallyl disulfide (DADS) induces the antitumorigenic NSAID-activated gene (NAG-1) by a p53-dependent mechanism in human colorectal HCT 116 cells. J. Nutr. 132, 773–778.

Callen, D.F., Baker, E., Simmers, R.N., Seshadri, R., Roninson, I.B., 1987. Localization of the human multiple drug resistance gene, MDR1, to 7q21. 1. Hum. Genet. 77, 142–144.

Chang, X.B., Hou, Y.X., Riordan, J.R., 1997. ATPase activity of purified multidrug resistance-associated protein. J. Biol. Chem. 272, 30962–30968.

Chao, C.C., Ma, C.M., Lin-Chao, S., 1991. Co-amplification and over-expression of two mdr genes in a multidrug-resistant human colon carcinoma cell line. FEBS Lett. 291, 214–218.

Chiang, J.H., Yang, J.S., Ma, C.Y., Yang, M.D., Huang, H.Y., Hsia, T.C., et al., 2011. Danthron, an anthraquinone derivative, induces DNA damage and caspase cascades-mediated apoptosis in SNU-1 human gastric cancer cells through mitochondrial permeability transition pores and Bax-triggered pathways. Chem. Res. Toxicol. 24, 20–29.

Demeule, M., Brossard, M., Turcotte, S., Regina, A., Jodoin, J., Beliveau, R., 2004. Diallyl disulfide, a chemopreventive agent in garlic, induces multidrug resistance-associated protein 2 expression. Biochem. Biophys. Res. Commun. 324, 937–945.

Fan, C.W., Chan, C.C., Chao, C.C., Fan, H.A., Sheu, D.L., Chan, E.C., 2004. Expression patterns of cell cycle and apoptosis-related genes in a multidrug-resistant human colon carcinoma cell line. Scand. J. Gastroenterol. 39, 464–469.

Filomeni, G., Aquilano, K., Rotilio, G., Ciriolo, M.R., 2003. Reactive oxygen species-dependent c-Jun NH2-terminal kinase/c-Jun signaling cascade mediates neuroblastoma cell death induced by diallyl disulfide. Cancer Res. 63, 5940–5949.

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

Goldstein, L.J., Galski, H., Fojo, A., Willingham, M., Lai, S.L., Gazdar, A., et al., 1989. Expression of a multidrug resistance gene in human cancers. J. Natl. Cancer Inst. 81, 116–124.

Grant, C.E., Kurz, E.U., Cole, S.P., Deeley, R.G., 1997. Analysis of the intron-exon organization of the human multidrug-resistance protein gene (MRP) and alternative splicing of its mRNA. Genomics 45, 368–378.

Harnett, P.R., Tattersall, M.H., Coates, A.S., van Couten, R., Forbes, J., 1987. Drug resistance in clinical practice: patterns of treatment failure in patients receiving systemic therapy for advanced breast cancer. Eur. J. Cancer Clin. Oncol. 23, 1601–1605.

Ho, Y.T., Yang, J.S., Lu, C.C., Chiang, J.H., Li, T.C., Lin, J.J., et al., 2009. Berberine inhibits human tongue squamous carcinoma cancer tumor growth in a murine xenograft model. Phytomedicine 16, 887–890.

Hong, Y.S., Ham, Y.A., Choi, J.H., Kim, J., 2000. Effects of allyl sulfur compounds and garlic extract on the expression of Bcl-2, Bax, and p53 in non small cell lung cancer cell lines. Exp. Mol. Med. 32, 127–134.

Ibrahim, S.S., Nassar, N.N., 2008. Diallyl sulfide protects against N-nitrosodiethylamine-induced liver tumorigenesis: role of aldose reductase. World J. Gastroenterol. 14, 6145–6153.

Ji, B.C., Hsu, W.H., Yang, J.S., Hsia, T.C., Lu, C.C., Chiang, J.H., et al., 2009. Gallic acid induces apoptosis via caspase-3 and mitochondrion-dependent pathways in vitro and suppresses lung xenograft tumor growth in vivo. J. Agric. Food Chem. 57, 7596–7604.

Kalayarasan, S., Prabhu, P.N., Sriram, N., Manikandan, R., Arumugam, M., Sudhandiran, G., 2009. Diallyl sulfide enhances antioxidants and inhibits inflammation through the activation of Nrf2 against gentamicin-induced nephrotoxicity in Wistar rats. Eur. J. Pharmacol. 606, 162–171.

Koizumi, K., Iwasaki, Y., Narukawa, M., Iitsuka, Y., Fukao, T., Seki, T., et al., 2009. Diallyl sulfides in garlic activate both TRPA1 and TRPV1. Biochem. Biophys. Res. Commun. 382, 545–548.

Kwon, K.B., Yoo, S.J., Ryu, D.G., Yang, J.Y., Rho, H.W., Kim, J.S., et al., 2002. Induction of apoptosis by diallyl disulfide through activation of caspase-3 in human leukemia HL-60 cells. Biochem. Pharmacol. 63, 41–47.

Lai, K.C., Hsu, S.C., Kuo, C.L., Yang, J.S., Ma, C.Y., Lu, H.F., et al., 2011. Diallyl sulfide, diallyl disulfide, and diallyl trisulfide inhibit migration and invasion in human colon cancer colo 205 cells through the inhibition of matrix metalloproteinase-2, -7, and -9 expressions. Environ. Toxicol.

Liem, A.A., Chamberlain, M.P., Wolf, C.R., Thompson, A.M., 2002. The role of signal transduction in cancer treatment and drug resistance. Eur. J. Surg. Oncol. 28, 679–684.

Lu, C.C., Yang, J.S., Huang, A.C., Hsia, T.C., Chou, S.T., Kuo, C.L., et al., 2010. Chrysophanol induces necrosis through the production of ROS and alteration of ATP levels in J5 human liver cancer cells. Mol. Nutr. Food Res. 54, 967–976.

Milner, J.A., 2001. A historical perspective on garlic and cancer. J. Nutr. 131, 1027S–1031S.

Nakagawa, H., Tsuta, K., Kiuchi, K., Senzaki, H., Tanaka, K., Hioki, K., et al., 2001. Growth inhibitory effects of diallyl disulfide on human breast cancer cell lines. Carcinogenesis 22, 891–897.

Park, E.K., Kwon, K.B., Park, K.I., Park, B.H., Jhee, E.C., 2002. Role of Ca(2+) in diallyl disulfide-induced apoptotic cell death of HCT-15 cells. Exp. Mol. Med. 34, 250–257.

Perez-Tomas, R., 2006. Multidrug resistance: retrospect and prospects in anti-cancer drug treatment. Curr. Med. Chem. 13, 1859–1876.

Singh, A., Shukla, Y., 1998. Antitumor activity of diallyl sulfide in two-stage mouse skin model of carcinogenesis. Biomed. Environ. Sci. 11, 258–263.

Slovak, M.L., Ho, J.P., Bhardwaj, G., Kurz, E.U., Deeley, R.G., Cole, S.P., 1993. Localization of a novel multidrug resistance-associated gene in the HT1080/DR4 and H69AR human tumor cell lines. Cancer Res. 53, 3221–3225.

Sundaram, S.G., Milner, J.A., 1996. Diallyl disulfide induces apoptosis of human colon tumor cells. Carcinogenesis 17, 669–673.

Wu, C.C., Sheen, L.Y., Chen, H.W., Tsai, S.J., 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.

Xiao, D., Choi, S., Johnson, D.E., Vogel, V.G., Johnson, C.S., Trump, D.L., et al., 2004. 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 23, 5594–5606.

Xiao, D., Herman-Antosiewicz, A., Antosiewicz, J., Xiao, H., Brisson, M., Lazo, J.S., et al., 2005. Diallyl trisulfide-induced G(2)-M phase cell cycle arrest in human prostate cancer cells is caused by reactive oxygen species-dependent destruction and hyperphosphorylation of Cdc 25 C. Oncogene 24, 6256–6268.

Xiao, D., Singh, S.V., 2006. Diallyl trisulfide, a constituent of processed garlic, inactivates Akt to trigger mitochondrial translocation of BAD and caspase-mediated apoptosis in human prostate cancer cells. Carcinogenesis 27, 533–540.

Yang, J.S., Chen, G.W., Hsia, T.C., Ho, H.C., Ho, C.C., Lin, M.W., et al., 2009. Diallyl disulfide induces apoptosis in human colon cancer cell line (COLO 205) through the induction of reactive oxygen species, endoplasmic reticulum stress, caspases casade and mitochondrial-dependent pathways. Food Chem. Toxicol. 47, 171–179.

Yin, M.C., Tsao, S.M., 1999. Inhibitory effect of seven Allium plants upon three Aspergillus species. Int. J. Food Microbiol. 49, 49–56.

Zhai, Q., Lu, S.R., Lin, Y., Yang, Q.L., Yu, B., 2008. Oxidative stress potentiated by diallylsulfide, a selective CYP2E1 inhibitor, in isoniazid toxic effect on rat primary hepatocytes. Toxicol. Lett. 183, 95–98.

Please cite this article in press as: Lai, K.-C., et al., Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells *in vitro* and *in vivo*. Phytomedicine (2012), doi:10.1016/j.phymed.2012.02.004