Downregulation of *GST*π expression by tryptanthrin contributing to sensitization of doxorubicin-resistant MCF-7 cells through c-jun NH_2-terminal kinase-mediated apoptosis

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Overexpression of *GST*π and underexpression of *Topo II* expression are associated with multidrug resistance (MDR) phenotype through nontransporter pathway. Tryptanthrin, a quinazoline derivative, was reported to sensitize resistant cells to doxorubicin by downregulation of *MDR1* expression. This study aims to extendedly investigate the effect of tryptanthrin on the role of nontransporter-based genes in determining the MDR response in doxorubicin-resistant MCF-7 cells (MCF-7/adr). Results show that tryptanthrin downregulates *GST*π expression and reduces glutathione S-transferase (GST) activity, but has no effect on *Topo II* expression. Less production of GSTπ decomposes the protein–protein interactions of GSTπ and c-jun NH_2-terminal kinase (JNK). The resulting free-form JNK undergoes phosphorylation upon elevated intracellular doxorubicin accumulation and subsequently activates JNK-mediated apoptosis. In conclusion, in addition to transporter pathway, tryptanthrin reverses MDR partly by modulating GSTπ-related pathway, a nontransporter pathway, in MCF-7/adr cells. It indicates that tryptanthrin may act as a potential chemoadjuvant agent through multiple targets. *Anti-Cancer Drugs* 20:382–388 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Multidrug resistance (MDR) involves a number of mechanisms, classified into two major categories: transporter-based and nontransporter-based MDR [1]. Transporter-based MDR mechanisms are mainly caused by the transport proteins of ATP-binding cassette family [2]. These membrane proteins actively transport drugs out of cells, resulting in a reduction of intracellular drug concentration. In contrast, nontransporter-based MDR is caused by altered activity of some enzyme systems such as glutathione S-transferases (GSTs), topoisomerases and apoptosis cascades [3].

GSTs serve two distinct roles in the development of drug resistance through detoxification and inhibition of the stress-activated protein kinases (SAPK). GSTs comprise an enzyme system for drug and xenobiotic detoxification *in vivo*. GSTs conjugate organic molecules with glutathione (GSH), resulting in polar molecules, to protect cells from reactive oxidant damage [4]. Many anticancer drugs are biotransformed by GSTs toward less-potent metabolites. GSTπ, a cytosolic GST isozyme, is overexpressed in several drug-resistant cell lines. Among these are doxorubicin-resistant MCF-7/adr cells that express elevated level of GSTπ as well as P-glycoprotein (P-gp) [5]. In addition to augmentation of GST activity, alteration of cellular GSH level also contributes to doxorubicin resistance [6]. Stress-activated signal transduction pathways mediated by c-jun NH_2-terminal kinase (JNK)/SAPK, playing an important role in both cell survival and cell death, are partly regulated by the protein–protein interactions with GSTπ [7–9]. In unstressed cells, low JNK activity is observed because of the sequestration of the protein in a GSTπ–JNK complex. Conjugation of doxorubicin with GSH changes conformation of the active center of GSTπ, enhancing the JNK-mediated apoptosis [10]. Some GST inhibitors have been designed for altering the regulation of mitogen-activated protein kinase pathway. Ethacrynic acid is an inhibitor of all classes of GST isozymes, clinically used in combination with thiotepa [11]. Terrapin 199, a glutathione analog-based GST inhibitor, has been modeled specifically to inhibit GSTπ [12].

Topoisomerases are also important targets of many anticancer drugs. Topoisomerase II (Topo II) is the therapeutic target of doxorubicin and etoposide for their cytotoxicity. Reduction of *Topo II* expression or gene mutation of *Topo II* has been shown in doxorubicin-resistant and etoposide-resistant cells [13]. Resistance

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may occur alone or concurrent to P-gp overexpression [14]. Correlation of downregulation of *Topo II* to MDR phenotype is still poorly understood.

Tryptanthrin is a bioactive ingredient of *Polygonum tinctoriu*, which belongs to the Indigo plant family. It is a quinazoline derivative with various biological activities such as antimicrobial, antitumor, and anti-inflammatory activities [15–17]. We previously found that tryptanthrin was able to reduce *MDR1* expression [18]. In this report, we extendedly investigate whether tryptanthrin acts on nontransporter sites to reverse MDR phenotype.

Materials and methods

Chemicals, cell lines, and cell culture

Tryptanthrin (6,12-dihydro-6,12-dioxoindolo-[2,1-b]quinazoline) was synthesized as described [18]. MCF-7/wt (wild-type) and MCF-7/adr (doxorubicin-resistant) cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and 100 ng/ml penicillin/streptomycin (Invitrogen, Carlsbad, California, USA) at 37°C in 5% CO_2. MCF-7/adr cells were grown in the presence of 6–8 μg/ml of doxorubicin, which was removed from the medium 1 week before each assay.

Cell viability assay

Cells were seeded into 96-well plates at a density of 5×10^3 cells/well. Cytotoxicity was determined by the MTT assay after incubation of cells with doxorubicin or tryptanthrin at various concentrations for 2 or 5 days, respectively. In the assay of doxorubicin plus tryptanthrin treatment, cells were preincubated with tryptanthrin for 5 days and then with doxorubicin for 2 days. Growth inhibition of 50% (IC_{50}) is determined at the drug concentration that results in 50% reduction of cell viability.

Determination of gene expression by reverse-transcriptase PCR and real-time PCR

Expression of *GSTπ*, *Topo IIα*, and *Topo IIβ* was assayed after 35 cycles of reverse-transcriptase (RT)-PCR as reported [19,20]. Total RNA was extracted from cells, followed by synthesis of the first strand cDNA using an Oligo dT (Promega, Madison, Wisconsin, USA) as primer. Primers used in this experiment are as follows:

(1) *GSTπ* primers (to generate a 137-bp fragment) sense 5′-CTCCGCTGCAAATACATCTC-3′ antisense 5′-ACAATGAAGGTCTTGCCTCC-3′.
(2) *Topoisomerase IIα* primers (to generate a 322-bp fragment) sense 5′-GGCTCGATTGTTATTTCCAC-3′ antisense 5′-GGTTGTAGAATTAAGAATAGC-3′.
(3) *Topoisomerase IIβ* primers (to generate a 304-bp fragment) sense 5′-GCTGTGGATGACAACCTCC-3′ antisense 5′-CTGTGTTTCTGTCCACTAC-3′.
(4) *GAPDH* primers (to generate a 122-bp fragment) sense 5′-AGTCAACGGATTTGGTCGTA-3′ antisense 5′-GGAACATGTAAACCATGTAG-3′.

RT-PCR products were separated by electrophoresis on a 2% agarose gel. Real-time PCR reactions were performed on the LightCycler system (Roche Diagnostics, Basel, Switzerland) using FastStart DNA Master SYBER green I kits. Primers used for real-time PCR were the same as that for RT-PCR. The relative amount of PCR products was calibrated by a standard curve from a series of dilutions of cDNA of the corresponding cell line. The quantitative measurement of each gene was normalized to the amount of GAPDH cDNA. To confirm amplification specificity, the PCR products were subjected to a melting curve analysis.

Immunoblot analysis

Cells were seeded into 6-well plates at a density of 2×10^5 cells/well in the presence of tryptanthrin at 10^{-6} mol/l for 5 days. To detect phosphorylation of JNK, tryptanthrin-treated cells were added with doxorubicin at 10^{-6} mol/l for various time intervals. Cells were trypsinized and cell pellets were washed twice with ice-cold PBS. Total cellular proteins (80 μg/lane) were separated by electrophoresis on 10% SDS-polyacrylamide gels and transferred to a polyvinylidene fluoride membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Proteins were labeled with primary antibody. Anti-human GSTπ (Chemicon, Temecula, California, USA), anti-p-JNK G7, and anti-proliferating cell nuclear antigen PC10 (Santa Cruz, Santa Cruz, California, USA), anti-β-actin AC15 (Sigma, St Louis, Missouri, USA), rabbit anti-Topo IIα and rabbit anti-Topo IIβ (Abcam, Cambridge, Massachusetts, USA) were used as primary antibodies. Immunoreactive bands were detected by anti-mouse horseradish peroxidase or anti-rabbit peroxidase-conjugated secondary antibody (Chemicon). Proteins were visualized via enhanced chemiluminescence (ECL detection kit, GE Healthcare). Densitometric analysis was performed by Molecular Dynamics (GE Healthcare).

Glutathione-S transferase activity assay

1-chloro-2,4-dinitrobenzene (CDNB) is a substrate of GST, used for GST enzyme activity assay. Upon conjugation of the thiol group of glutathione to CDNB, absorbance increases at 340 nm. Total GST enzyme activity was detected as described [21] with modification. Briefly, cells were treated with tryptanthrin for 1–5 days and then collected for centrifugation at 1000–2000*g* for 10 min at 4°C. Cell pellets were homogenized in cold buffer (100 mmol/l potassium phosphate, pH 7.0, containing 2 mmol/l EDTA). The cytosol (200 μg) was subjected to catalytic assay by incubation with 1 mmol/l glutathione and 1 mmol/l CDNB to form color

products detected by Spectra Max plus (Molecular Devices, Montreal, Canada).

Immunoprecipitation

Cell pellets were resuspended in lysis buffer and incubated on ice for 30 min. Lysates were then centrifuged at 14 000*g* for 15 min at 4°C and 300 μg of protein was incubated in lysis buffer with 10 μl of anti-JNK1 (F3) antibody (Santa Cruz) to a total volume of 300 μl for 2 h at 4°C. Immunocomplexes were absorbed with 20 μl of protein A-sepharose at 4°C over night. After three washes with lysis buffer, immune pellets were boiled in SDS sample buffer and subjected to electrophoresis on a 15% SDS-polyacrylamide gel, followed by immunoblotting and densitometry.

Apoptosis detected by flow cytometry using propidium iodide staining and caspase 3/7 assay

Cells were treated with tryptanthrin and/or 10^{-6} mol/l doxorubicin for 1–5 days, then harvested by 0.25% trypsin and washed with PBS. Cells (2×10^5) were fixed in 70% ice-cold EtOH/PBS for 20 min on ice, washed with PBS and incubated in PI solution (69 mmol/l PI, 388 mmol/l sodium citrate and 100 μg/ml RNase A). Then cells were analyzed immediately using an FACS Caliber (Beckton Dickinson, Franklin Lakes, New Jersey, USA). SubG1 population of the cell-cycle histogram was quantified.

Caspase 3/7 activity was measured using the Caspase-glo 3/7 assay kit (Promega, Madison, Wisconsin, USA). After treatment with tryptanthrin and/or 10^{-6} mol/l doxorubicin for 5 days, the cells were incubated with Caspase-glo reagent for 1 h in the dark, and the luminescence was measured with a luminometer (Berthold Technologies, Bad Wildbad, Germany). Equal numbers of cells were analyzed by counting a parallel set of cells and determining the total cell number for each sample.

Intracellular accumulation assay

Rhodamine 123 (Rh-123) is a substrate for P-gp and is widely used as an indicator for the activity of P-gp. MCF-7/adr cells were seeded into 6-well plates at a density of 2×10^5 cells/well. Cells were pretreated with 10^{-6} mol/l tryptanthrin for 5 days, and then incubated with 1 mg/ml of Rh-123 in the dark at 37°C in 5% CO_2 for 1 h. After Rh-123 accumulation, cells were trypsinized and the cell pellet was washed twice with ice-cold PBS. Then the cells were analyzed immediately using an FACS Caliber (Beckton Dickinson). Emission of green fluorescence by Rh-123 was measured using a 530 nm bandpass filter.

Statistical analysis

Data are presented as mean ± SD for the indicated number of separate experiments. Comparisons between groups were analyzed using Student's *t*-tests. Probability values of *P* less than 0.05 are considered statistically significant.

Table 1 IC_{50} values (in μmol/l) of doxorubicin and tryptanthrin in two cell lines

Cell line	Doxorubicin	Tryptanthrin	Doxorubicin + tryptanthrin (10^{-6} mol/l)
MCF-7/wt	0.14 ± 0.03	8.23 ± 0.37	0.09 ± 0.01
MCF-7/adr	11.13 ± 0.51	5.63 ± 0.53	0.76 ± 0.06

Cell viability was measured using the MTT assay.
Data are presented as mean ± SD of quadruplicate determinations. IC_{50}, growth inhibition of 50%.

Results

Effect of tryptanthrin on cell viability and doxorubicin sensitivity

Cell viability was first measured to confirm whether tryptanthrin alone was toxic to cells or not. The IC_{50} of tryptanthrin showed that the toxicity of tryptanthrin was mild in MCF-7/wt and MCF-7/adr cells (Table 1). MCF-7/adr showed an 80-fold increase in resistance to doxorubicin (IC_{50} from 0.14 to 11.13 μmol/l), compared with MCF-7/wt, using MTT assay. Tryptanthrin at 10^{-6} mol/l could markedly enhance cytotoxicity of doxorubicin in MCF-7/adr. A 12-fold decrease in doxorubicin IC_{50} (from 9.13 to 0.76 μmol/l) was noted in MCF-7/adr.

Tryptanthrin downregulates *GST*π expression

Several mechanisms underlying MDR include overexpression of *MDR1* and *GST*π genes and underexpression of topoisomerases. Besides inhibiting *MDR1* gene expression upon tryptanthrin treatment in MCF-7/adr cells, expression of *GST*π, *Topo IIα*, and *Topo IIβ* genes was extendedly detected and quantified by RT-PCR and real-time PCR. In Fig. 1, *GST*π mRNA level was elevated only in MCF-7/adr and the expression of *Topo IIα* and *IIβ* in MCF-7/adr decreased, as compared with that in MCF-7/wt. According to real-time PCR results, *GST*π expression was largely suppressed when MCF-7/adr cells were treated with tryptanthrin (Fig. 1b). Reduction of GSTπ protein was accompanied (Fig. 1c). However, expression of *Topo IIα* and *IIβ* did not change with tryptanthrin treatment.

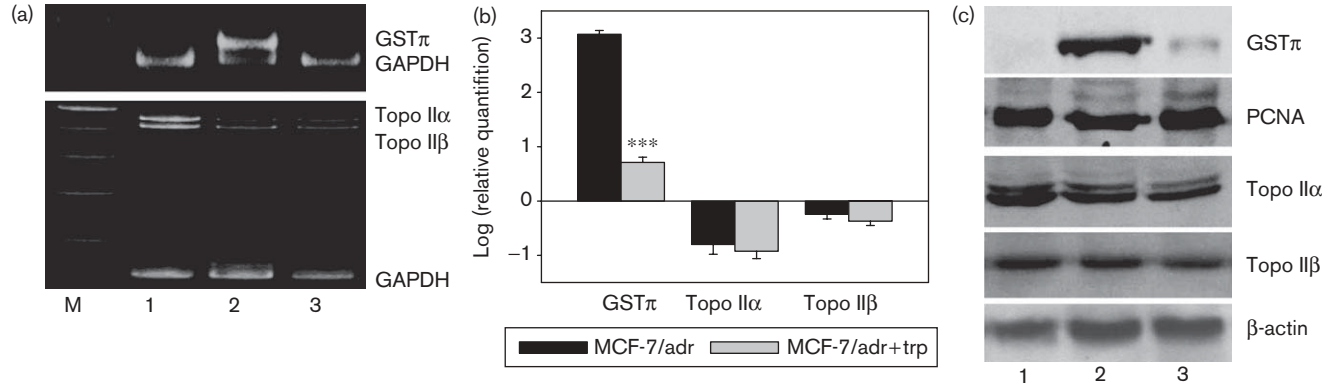
Glutathione-S transferase activity diminished by tryptanthrin in MCF-7/adr cells

As *GST*π mRNA and protein levels were suppressed by tryptanthrin, we determined if *GST*π enzyme activity would decline in MCF-7/adr cells with tryptanthrin. Total GST enzyme activity assay is commonly used to detect the alteration of substrate-selective GST isozyme activity. Results showed that GST enzyme activity markedly decreased with time in MCF-7/adr cells treated with tryptanthrin (Fig. 2a).

Tryptanthrin treatment decomposes GSTπ–JNK complex

GSTπ is an inhibitor of JNK by forming the GSTπ–JNK complex through protein–protein interaction. Therefore, we examined whether the reduced GSTπ was related to

Fig. 1



Gene expression and protein synthesis of *GSTπ* and *Topo II* were suppressed in MCF-7/adr cells treated with 10^{-6} mol/l tryptanthrin (trp). (a) mRNA levels were detected in MCF-7/wt (lane 1), MCF-7/adr (lane 2) cells and MCF-7/adr cells with tryptanthrin (lane 3). Total cellular RNAs were isolated and followed by reverse-teranscriptase-PCR to generate the 137 bp *GSTπ*, 322 bp *Topo IIα*, and 304 bp *Topo IIβ* cDNA fragments, then were separated by electrophoresis on 2% agarose gels. GAPDH was used as the internal control (122 bp). Data are representative of three independent experiments. (b) Real-time PCR was performed using the same primers as in (a). Expression of *GSTπ*, *Topo IIα*, and *Topo IIβ* was measured in MCF-7/adr with or without tryptanthrin treatment. The values of relative quantification of each sample were calculated by the corresponding expression in MCF-7/wt cells. Data are represented as mean ± SD ($n=3$). ***$P<0.001$. (c) Protein levels of MCF-7/wt (lane 1), MCF-7/adr (lane 2), and MCF-7/adr with tryptanthrin (lane 3) were detected using immunoblotting. Proliferating cell nuclear antigen (PCNA) for GSTπ and β-actin for Topo IIαβ were used as the loading controls. Data are representative of three independent experiments.

the amount of free-form JNK1, which could trigger apoptosis under stress [9,22]. Immunoprecipitation was performed with anti-JNK1 and was followed by western blot analysis in MCF-7/wt or MCF-7/adr cells. After the cells were treated with 10^{-6} mol/l tryptanthrin for 5 days, the total amount of immunoblotted JNK1 was not significantly altered, but the amount of coprecipitated GSTπ decreased significantly in MCF-7/adr. The decrease in intensity of the immunoreactive band from tryptanthrin-treated MCF-7/adr showed a statistically significant value of $P = 0.002$ (Fig. 2b).

We then examined the phosphorylation state of JNK in the presence of tryptanthrin and/or doxorubicin in MCF-7/adr cells. JNK was less phosphorylated in MCF-7/adr without/or with tryptanthrin (Fig. 2c, lanes 1 and 2). Tryptanthrin alone could not activate JNK through phosphorylation. Until 8 h after the addition of doxorubicin, the phosphorylated form of JNK dramatically increased (Fig. 2c, lane 4).

Apoptosis enhanced by tryptanthrin and doxorubicin cotreatment in MCF-7/adr cells

To test whether the appearance of the phosphorylated form of JNK was closely correlated with the development of apoptosis, the DNA contents of MCF-7/adr cells in the presence of tryptanthrin were determined by flow cytometric analysis and caspase 3/7 activity was assayed. MCF-7/adr cells treated with 10^{-6} mol/l tryptanthrin or 10^{-6} mol/l doxorubicin alone for 5 days showed equally regular levels of DNA contents (Fig. 3a) and equally low caspase activity (Fig. 3b). Nevertheless, when cells were treated with 10^{-6} mol/l doxorubicin in combination with 10^{-6} mol/l tryptanthrin for 5 days, the subG$_1$ population of MCF-7/adr cells was six-fold higher than that treated with tryptanthrin or doxorubicin alone. A large increase of caspase 3/7 activity was also observed in MCF-7/adr cells upon cotreatment with tryptanthrin and doxorubicin for 5 days.

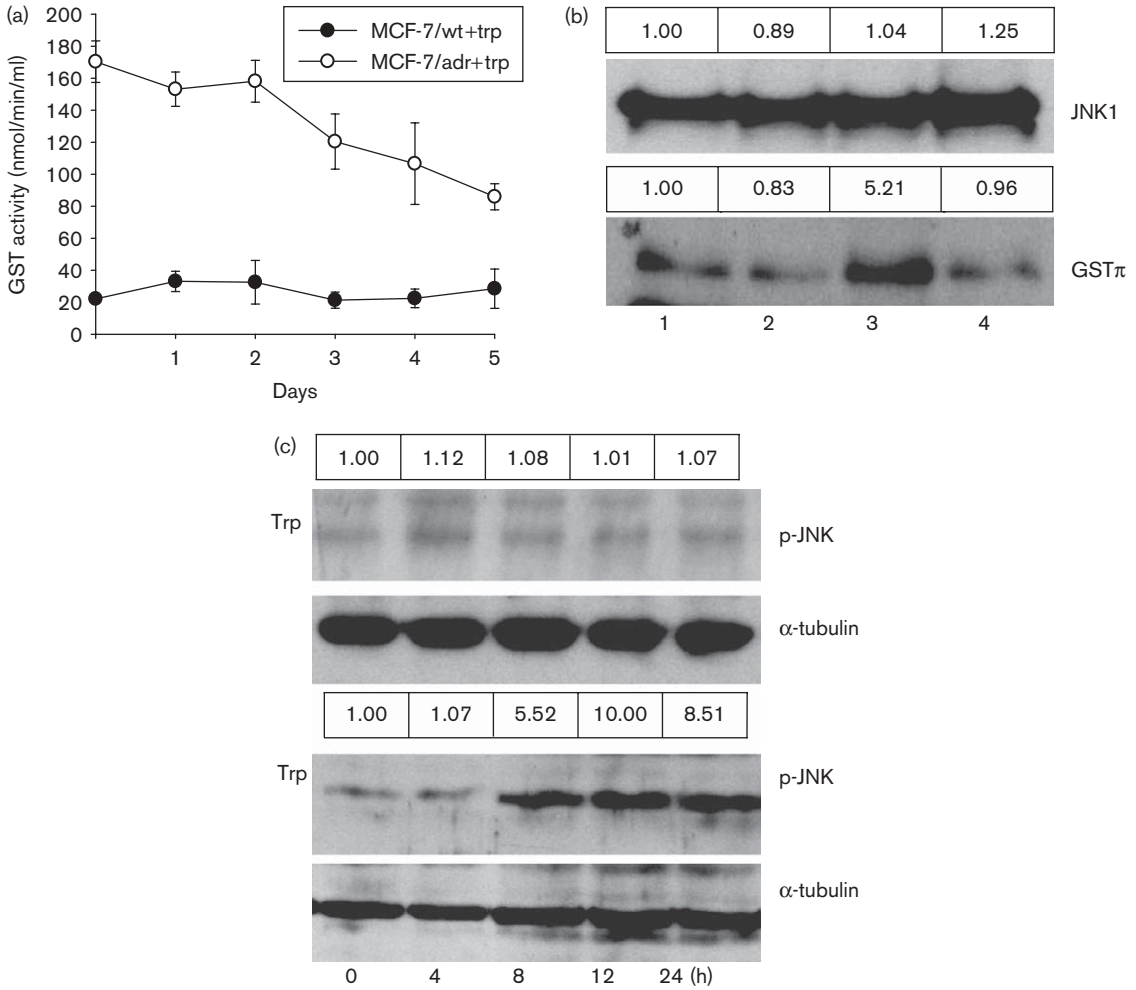
Increases in intracellular doxorubicin upon tryptanthrin treatment in MCF-7/adr cells

To investigate the importance of doxorubicin in JNK-mediated apoptosis affected by tryptanthrin, Rh-123 was used for indirect assay of drug accumulation. MCF-7/adr cells were preincubated with tryptanthrin 10^{-6} mol/l for 5 days and then incubated with Rh-123. Low intracellular fluorescence was significantly reversed to high-level counts in the presence of tryptanthrin in MCF-7/adr cells, which present overexpressed P-gp (Fig. 4). In control MCF-7 cells, the uptake of Rh-123 was not evidently influenced by tryptanthrin.

Discussion

Development of adjuvant agents to circumvent MDR is a new trend in cancer chemotherapy. Numerous strategies have been proposed to target a variety of sites for reversing MDR [23]. Among these approaches, targets for either transporter or nontransporter mechanisms were concerned. An ideal MDR-reversing agent would be able to act on more than one MDR-reversing mechanism so that drug-resistant cancer cells will not easily escape from

Fig. 2

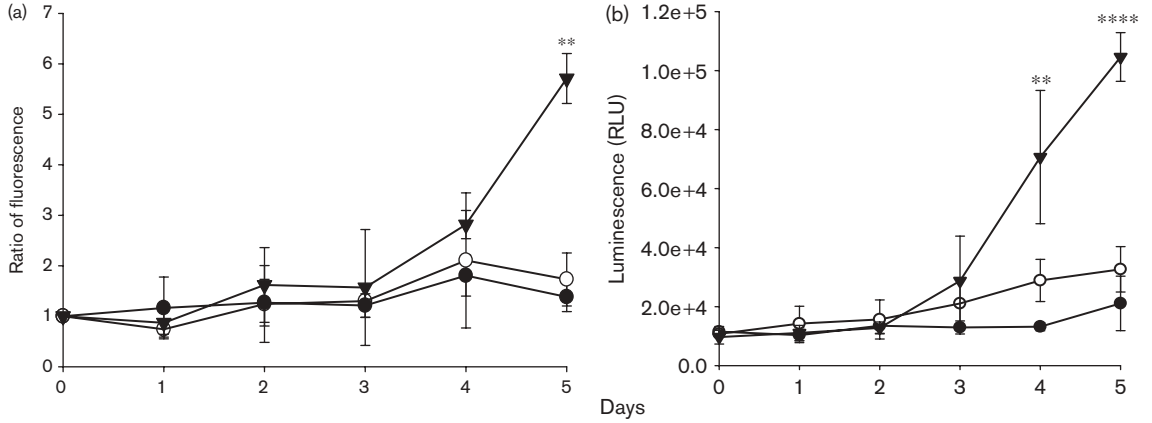


c-jun NH_2-terminal kinase (JNK) phosphorylation was enhanced by less GSTπ protein upon tryptanthrin treatment. (a) Reduction of glutathione S-transferase (GST) activity in MCF-7/adr cells treated with tryptanthrin. MCF-7/wt and MCF-7/adr cells were treated with 10^{-6} mol/l tryptanthrin for 1–5 days. Total GST enzyme activity was assessed by formation of thiol-conjugated dichloronitrobenzene. Data are represented as mean ± SD of three independent experiments. (b) Immunoprecipitation of total JNK and co-immunoprecipitated (co-IP) GSTπ. After treated with tryptanthrin, cell homogenates were immunoprecipitated with anti-JNK1 and subsequently immunoblotted with anti-JNK1 or anti-GSTπ. Total JNK and co-IP GSTπ in MCF-7/wt (lane 1), MCF-7/wt with tryptanthrin (lane 2), MCF-7/adr (lane 3), and MCF-7/adr with tryptanthrin (lane 4) were detected using immunoblotting. The intensity of the immunoreactive bands was measured by a densitometer and shown as indicated. (c) Phosphorylation of JNK (p-JNK) detected by immunoblotting. MCF-7/adr cells were treated with (lower) or without (upper) 10^{-6} mol/l tryptanthrin for 5 days. 10^{-6} mol/l doxorubicin (Dox) was subsequently added for various time intervals. α-tubulin was used as the loading control and the intensity of the immunoreactive bands was as indicated. Data are representative of three independent experiments in (b) and (c).

the synergistic effects of the MDR-reversing agent in chemotherapy. In this study, tryptanthrin may be one of the candidates.

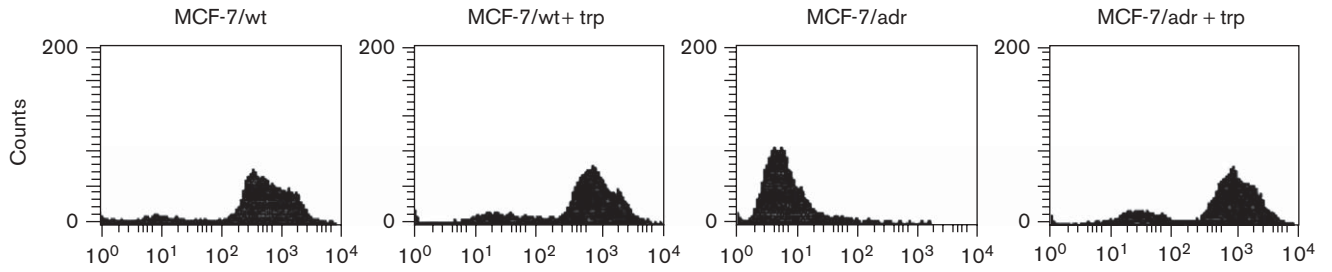
GSTs are overexpressed in some cancers and a variety of resistance-expressing cancer cells. Approaches targeting GSTs and GSH have been paid attention to treat cancers and attenuate MDR phenotype. GSTs, undergoing phase II metabolic reaction, can directly detoxify xenobiotics or drugs to develop drug resistance. Some anticancer agents such as mephalan and chlorambucil are substrates of GSTs and can be directly inactivated by catalytic conjugation to GSH through thioether bond formation [22]. Decrease of either GST activity or GSH level retards the biotransformation of anticancer drugs and enhances the efficacy of chemotherapy. Agents such as ethacrynic acid and prostaglandin analogs block GST activity and sensitize chlorambucil or mephalan [24,25]. However, some anticancer agents such as doxorubicin and mitomycin C are not direct substrates of GSTs. Detoxification of these two drugs is less likely to be the major cause of drug resistance. Studies have shown a regulatory role of GSTπ in the MAP kinase pathway, associated with drug resistance. GSTπ was reported to be the inhibitor of JNK by forming the GSTπ–JNK complex through protein–protein interactions [8]. The restricted JNK

Fig. 3



Doxorubicin-induced apoptosis was enhanced by tryptanthrin. (a) DNA fragmentation increased upon cotreatment with tryptanthrin and doxorubicin. MCF-7/adr cells were exposed to 10^{-6} mol/l doxorubicin (○), tryptanthrin (●) or 10^{-6} mol/l doxorubicin plus 10^{-6} mol/l tryptanthrin (▼) for 1–5 days in a complete medium, stained with PI, and then analyzed by flow cytometry. The *y*-axis is the ratio of fluorescence measured in tryptanthrin-treated versus untreated cells. Data are represented as mean ± SD ($n=4$). (**$P<0.005$; day 5 vs. day 0). (b) Assay of caspase-3/7 activity. MCF-7/adr cells were exposed to 10^{-6} mol/l doxorubicin (○), tryptanthrin (●) or 10^{-6} mol/l doxorubicin plus 10^{-6} mol/l tryptanthrin (▼) for 1–5 days and subjected to casepase-3/7 assay. Data are represented as mean ± SD ($n=4$). (**$P<0.005$, ****$P<0.00005$; day 5 vs. day 0).

Fig. 4



Increased intracellular drug accumulation in tryptanthrin (trp)-treated MCF-7/adr cells. Accumulation of Rh-123 was measured 5 days after exposure to 10^{-6} mol/l tryptanthrin in MCF-7/wt or MCF-7/adr cells. The *x*-axis is the fluorescence intensity, and the *y*-axis is the cell count. Data are representative of three independent experiments.

activity is correspondent to the suppression of JNK-mediated apoptosis, which contributes to the development of drug resistance. This process provides an explanation for the numerous anticancer drugs that are not substrates for GST of drug resistance linking GST overexpression [22].

A variety of GST inhibitors were shown to modulate drug resistance by sensitizing cancer cells to anticancer agents. Most of them act as inhibitors by reacting with GSTπ or forming a conjugate with GSH. For example, a conjugate of doxorubicin with GSH expresses potent cytotoxicity by suppression of GSTπ activity in hepatoma cells [26]. An anti-inflammatory drug, sulfasalazine, reduces GSH levels and enhances cytotoxicity of doxorubicin in breast cancer cells [27]. In this study, unlike the known GST inhibitors, tryptanthrin acts in a different way to regulate *GSTπ* expression. Data show that tryptanthrin suppresses *GSTπ* gene expression and reduces production of RNA and protein in MCF-7/adr cells. GST activity decreases with the suppression of *GSTπ* expression. The decrease of the GSTπ protein by tryptanthrin treatment may dissociate the GSTπ–JNK complex, resulting in free-form JNK which is susceptible to phosphorylation activation upon doxorubicin treatment, followed by triggering JNK-mediated apoptosis shown as a marked increase in $subG_1$ population and caspase 3/7 activity. Like cisplatin and carboplatin which are examples of drugs whereby JNK activity is required for potentiation of cytotoxicity [28], doxorubicin is speculated to activate free-form JNK and the downstream transcription factor c-jun by phosphorylation to turn on apoptosis cascades [29]. After MCF-7/adr cells are treated with tryptanthrin, a pronounced increase in intracellular doxorubicin level, with indirect

evidence of Rh-123 accumulation, supports the requirement of doxorubicin in JNK activation. With regard to no detectable difference in the amount of immunoprecipitated JNK1 after tryptanthrin treatment, it is likely that tryptanthrin simply acts on downregulation of *GSTπ* expression, but has less effect on JNK expression, to modulate the interchange of bound and free forms of JNK.

Topo II in tumor cells is the target enzyme of some anticancer agents. If Topo II activity decreases or a *Topo II* gene mutation develops, Topo II-related MDR results [30]. Our data show that *Topo IIα* and *IIβ* expression decrease in MCF-7/adr cells. However, tryptanthrin does not affect Topo IIα and IIβ mRNA and protein levels in MCF-7/adr cells, indicating that *Topo IIα* and *IIβ* genes were less likely to be the targets of tryptanthrin for MDR reversal activity.

In conclusion, we showed that tryptanthrin could downregulate *GSTπ* gene, accompanied by less GST activity, to partly confer its MDR-reversing effect in doxorubicin-resistant cells. Combined with a pronounced increase in intracellular doxorubicin owing to the diminished P-gp expression, tryptanthrin is an important contributor involving in modulating the amount of free-form JNK in doxorubicin-required JNK/c-jun apoptosis pathway.

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