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ORIGINAL ARTICLE

Synergistic interaction between tetrandrine and chemotherapeutic agents and influence of tetrandrine on chemotherapeutic agent-associated genes in human gastric cancer cell lines

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Abstract
Purpose Tetrandrine (Tet), a bis-benzylisoquinoline alkaloid that was isolated from the dried root of Hang-Fang-Chi (*Stephania tetrandra S. Moore*), is well known as processing a marked antitumor effect in vitro and in vivo. The aim of this study was to assess the interaction between tetrandrine and chemotherapeutic agents widely used in gastric cancer treatment, and to investigate the influence of tetrandrine on chemotherapeutic agent-associated gene expression and apoptosis.
Methods Synergistic interaction on human gastric cancer BGC-823 and MKN-28 cells was evaluated using the combination index (CI) method. The double staining with both Annexin-V-FITC and PI was employed to distinguish the apoptotic cells from living cells. Expression of chemotherapeutic agent-associated genes, i.e., excision repair cross-complementing 1 (ERCC1), thymidylate synthase (TS), class III β-tubulin (β-tubulin III) and tau, of BGC-823 cells with or without tetrandrine treatment were measured by real-time quantitative PCR.
Results Tetrandrine had a synergistic effect on the cytotoxicity of chemotherapeutic agents in both two gastric cancer cell lines. The combination of tetrandrine and chemotherapeutic agents could also induce apoptosis in a synergistic manner. Tetrandrine could suppress the mRNA expression of ERCC1, TS, β-tubulin III and tau. Most prominently, ERCC1, TS and β-tubulin III mRNA levels were markedly suppressed at 0.29-, 0.12- and 0.60-fold, respectively, by the presentation of tetrandrine.
Conclusion Tetrandrine appears a promising candidate for combining with three chemotherapeutic agents. The possible mechanisms might be the synergistic apoptotic effect and the downregulation of chemotherapeutic agent-associated genes.

Keywords Synergy · Tetrandrine · Chemotherapeutic agents · Combination index · Gastric cancer

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Introduction

Gastric cancer is second leading cause of cancer death worldwide and continues to carry a poor prognosis, making it a therapeutic challenge for oncologists. Currently, it remains unclear to agree with the standard care, and even it differs in each country. Platinum compounds, 5-fluorouracil and taxanes might be considered the mainstay of chemotherapy for treatment of gastric cancer. Complete responses with single-agent therapy are uncommon, and partial response ranged 10–20% [1]. Antineoplastic agents often achieve antitumor activity at the expense of close to unacceptable toxicity. Various attempts have been made to improve the objective response rate to chemotherapy, including chemotherapeutic agents in combination, but the optimal combination regimen has remained elusive, possibly until now [2]. The rationale for combination two or more therapeutic agents is to achieve lower drug doses,

reduce toxicity and minimize or delay the emergence of resistance by target cells, and to identify potential synergistic effect. Some compound derived from Chinese medicine has been used as an adjunct to chemotherapy, demonstrating a great satisfactory prospective role in sensitization to chemotherapy [3].

Tetrandrine (Tet) is one member of bis-benzylisoquinoline alkaloid which was accepted as cytotoxic agent and fulfills certain structural requirements for antitumor activity [4]. It was isolated from the dried root of Hang-Fang-Chi (*Stephania tetrandra S. Moore*), and possessed a remarkable pharmacological profile, such as anti-inflammation [5], antioxidant [6] and antifibrotic [7]. It also elicits cytotoxic effect on cancer cells [8] and enhances the cytotoxicity of drug affected by multidrug resistance (MDR) via modulation of p-glycoprotein (P-gp) [9]. However, its potential role in cancer therapy has not been clearly addressed.

This preclinical study was therefore undertaken to investigate whether combination of tetrandrine and a series of chemotherapeutic agents resulted in a marked synergistic anticancer activity against gastric cancer cells. Those anticancer drugs tested, were selected for their differing modes of action and wide usage in gastric cancer chemotherapy. To elucidate further the mechanisms possibly involved in this action, we also investigated apoptosis induced by tetrandrine and chemotherapeutic agents singly and in combination and the influence of tetrandrine on expression of chemotherapeutic agent-associated genes.

Materials and methods

Cell lines and cell culture

Human well-differentiated gastric cancer MKN-28 cells and poorly differentiated BGC-823 cells were obtained from Shanghai Institute of Cell Biology (Shanghai, China). All cell lines were propagated in RPMI 1640 medium (GIBCO BRL), supplemented with 10% bovine serum, penicillin (100 U/ml)-streptomycin (100 μg/ml), pyruvate, glutamine and insulin at 37°C in a water-saturated atmosphere with 5% CO_2.

Drugs

Tetrandrine (molecular formula $C_{38}H_{42}N_2O_6$) was obtained as a powder with a purity of >98% from Jiangxi Yibo Pharmaceutical Development Company (Jiangxi, China). 5-fluorouracil (5-FU), oxaliplatin (Oxa) and docetaxel (Doc) were supplied from Jiangsu Hengrui Medicine Company (Jiangsu, China). All of the reagents were prepared extemporaneously in complete culture medium immediately prior to use in vitro. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from Sigma Chemical Company (St Louis, MO, USA). All other chemicals used were of the highest pure grade available.

Measurement of IC_{50}s for tetrandrine and chemotherapeutic agents

Tumor cells growing in log-phase were trypsinized and seed at 2×10^3 cells per well into 96-well plates and allowed to attach overnight. Medium in each well was replaced with fresh medium or medium with various concentrations of drugs in at least five replicate wells and left contact for 72 h. The medium-containing drug was decanted and the IC_{50} doses of each drug were determined by MTT assay described below. The IC_{50} was defined as the concentration required for 50% inhibition of cell growth. Each experiment was allocated ten wells containing drug-free medium for the control and performed on at least three separated occasions.

Determination of synergism and antagonism

Subconfluent gastric cancer cells were seeded at 2×10^3 cells/well in 96-well plates. Drugs were added either concomitantly or sequentially with six different concentrations of the single agents and six different concentrations of both agents at their fixed ratio based on their respective individual IC_{50} values for 72 h. The fractional inhibition of cell proliferation was calculated by comparison to control cultures. Dose–response curves were obtained for each drugs, and for multiple dilutions of a fixed-ratio combination of the two drugs.

Median effect analysis using the combination index (CI) method of Chou and Talalay [10] was employed to determine the nature of the interaction observed between tetrandrine and chemotherapeutic agents. The CI is defined by the following equation: $CI = (D)_1/(Dx)_1 + (D)_2/(Dx)_2 + \alpha(D)_1(D)_2/(Dx)_1(Dx)_2$, in which $(Dx)_1$ and $(Dx)_2$ are the concentrations for D_1 (tetrandrine) and D_2 (chemotherapeutic agent) alone that gives x% inhibition, whereas $(D)_1$ and $(D)_2$ in the numerators are the concentrations of tetrandrine and another drug that produce the identical level of effect in combination. $\alpha = 0$ when the drugs are mutually exclusive (i.e., with similar modes of action), while $\alpha = 1$ of they are mutually non-exclusive (i.e., with independent modes of action). CIs > 1 indicate antagonism, CIs < 1 indicate synergy, and CIs = 1 indicate

Table 1 The IC_{50} doses of tetrandrine and chemotherapeutic agents

Cell line	IC_{50} (mean ± SD, μm)			
	Tetrandrine	5-Fluorouracil	Oxaliplatin	Docetaxel
BGC-823	12.28 ± 1.15	32.23 ± 1.98	12.27 ± 0.77	0.05 ± 0.01
MKN-28	3.55 ± 0.38	5.88 ± 0.31	9.16 ± 0.58	14.86 ± 4.48

additivity. Each CI ratio represented here is the mean value derived from at least three independent experiments.

Cytotoxicity assay

The in vitro drug-induced cytotoxic effects were measured by the MTT reduction assay [11]. After treatment, 1/10 volume of MTT was added to each well, and the plate was further incubated at 37°C for another 4 h. Two hundred microliter DMSO was added to each well to solubilize the MTT-formazan product after removal of the medium. Absorbance at 570 nm was measured with a multiwell spectrophotometer (BioTek, VT, USA). Growth inhibition was calculated as a percentage of the untreated controls, which were not exposed to drugs.

Apoptosis assay

Cells were cultured in a 60-mm Petri disk and allowed to grow to 75–80% confluency. They were exposed to tetrandrine and anticancer durgs added singly or in combination for 48 h and compared with control cells not treated with drugs. Then they were collected and incubated with Annexin-V-FITC (Bender Medsystems, Burlingame, CA, USA) for determining surface exposure of phosphatidyl serine in apoptotic cells. Analyses were performed with a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA, USA).

Quantitative RT-PCR

BGC-823 cells were seeded on 6-well plates and treated with tetrandrine at its IC_{50} value for 72 h and harvested with trypsin, washed with PBS, and collected by centrifugation at 1,000 rpm for 5 min. Total RNA was extracted using Trizol reagent (Invitrogen, CA, USA) following the manufacture's protocol. cDNA was generated with random primers and the target cDNA sequences were amplified by quantitative PCR in a fluorescent temperature cycler (Mx3000P Real Time PCR System, Stratagene). Briefly, total RNA 1 μg was used for each RT reaction. The 20 μl PCR reaction mixture contained 1× primers and probe mixture [Applied Biosystems, Foster city, CA. Assay IDs: Hs00157415_m1 (ERCC1); Hs00426591_m1 (TS); Hs00964965_m1 (β-tubulin III); Hs00213491_m1 (tau); Hs99999903_m1(β-actin)], 1× Absolute QPCR Mix (ABgene, Surrey, UK). The PCR conditions were 50°C for 2 min, 95°C for 15 min, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min.

Relative gene expression quantifications were calculated according to the comparative Ct method using β-actin as an endogenous control and cells without tetrandrine treatment as calibrators. Final results were determined by the formula $2^{-\Delta\Delta Ct}$ [12] and were analysed with the Stratagene analysis software.

Statistical methods

Statistical comparisons were performed using Student's *t* test.

Results

Cytotoxicities of tetrandrine and chemotherapeutic agents against BGC-823 and MKN-28 cells

We first examined the cytotoxicity of each drug for BGC-823 and MKN-28 cells lines. As expected, tetrandrine and each chemotherapeutic agent individually increased the cytotoxicity of both two cell lines in a dose-dependent fashion. Table 1 shows the IC_{50} doses for both BGC-823 and MKN-28 cells lines following exposure to tetrandrine or chemotherapeutic agents. The response of BGC-823 cells to these drugs (except docetaxel, discussed additionally below) tended to be weaker than that of MKN-28 cells, suggesting the genetic make-up of the cells plays an important role in the response to drug treatment. The IC_{50} concentrations were then used to generate fixed ratios for subsequent combination studies and for the calculation of combination indices (CIs).

Effects of combination of tetrandrine and chemotherapeutic agents

To explore whether tetrandrine could enhance the effects of the chemotherapeutic agents currently used to treat gastric cancer, the effects of 72-h treatment

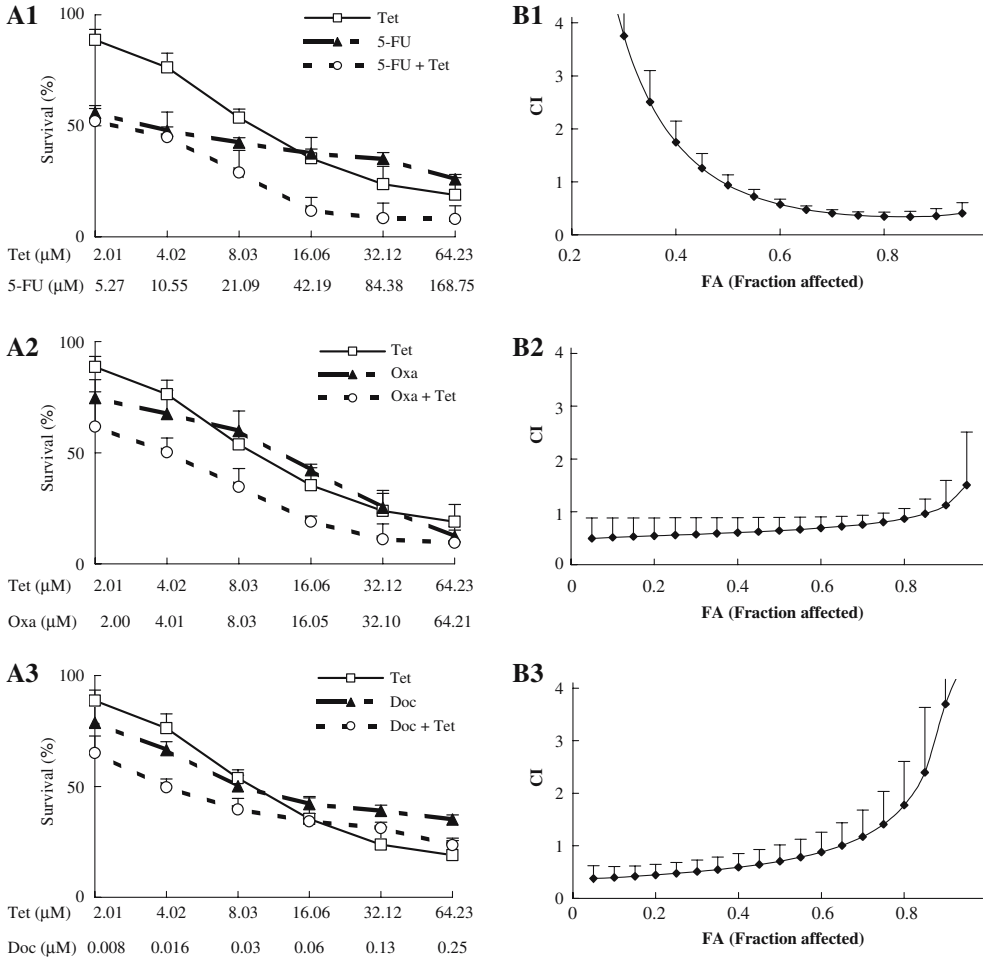

Fig. 1 Analysis of synergy between tetrandrine and 5-fluorouracil/oxaliplatin/docetaxel for BGC-823 cells. **a** Dose–response curve of tetrandrine and chemotherapeutic agents for BGC-823 cells. Data point, means of at least three independent experiment; *bars*, SD; **b** CI values at different level of growth inhibition effect (fraction affected, *FA*). Data point, means of at least three independent experiment; *bars*, SD. **a1**, **b1** tetrandrine plus 5-fluorouracil; **a2**, **b2** tetrandrine plus oxaliplatin; **a3**, **b3** tetrandrine plus docetaxel

with tetrandrine, 5-fluorouracil, oxaliplatin and docetaxel singly and in combination were examined. The combination ratios were designed to approximate the IC_{50} ratios of the individual component compounds, so that the contribution of anti-proliferative effect for each compound in the combinations is roughly the same [13].

Figures 1 and 2 show the dose–response curves for BGC-823 (Fig. 1a) and MKN-28 (Fig. 2a) cell lines exposed to tetrandrine and chemotherapeutic agents singly and in combination. For both cell lines, drug combinations gave a more decrease of cell survivals. To fully evaluate the nature of the interaction between tetrandrine and chemotherapeutic agents, we analysed the combination of both drugs using media-effect analysis, which resolves the degree of synergy, additivity, or antagonism at various levels of cell death.

We failed to detect the combination of tetrandrine and docetaxel on MKN-28 cells because the effect of docetaxel on MKN-28 cells shows a weak linear relationship (the linear correlation coefficient < 0.9) and weak reproducibility, indicating the inconformity of the data to the median-effect principle.

Figures 1 and 2 also illustrate the multiple drug effect obtained for BGC-823 (Fig. 1b) and MKN-28 (Fig. 2b) cells, respectively, which were treated simultaneously with tetrandrine and anticancer drugs and represented as fractional cell growth inhibition (FA) as a function of the CI. The results are summarized in Table 2, which shows, for each combination, the computer-calculated CI for 20, 50 and 80% cytotoxicity (Fa = 0.2, 0.5, 0.8, respectively). It was different in some extent of concentrations between two cell lines. But in all combinations, the CI values were below 1 at Fa = 0.5, indicating a synergistic anti-proliferative

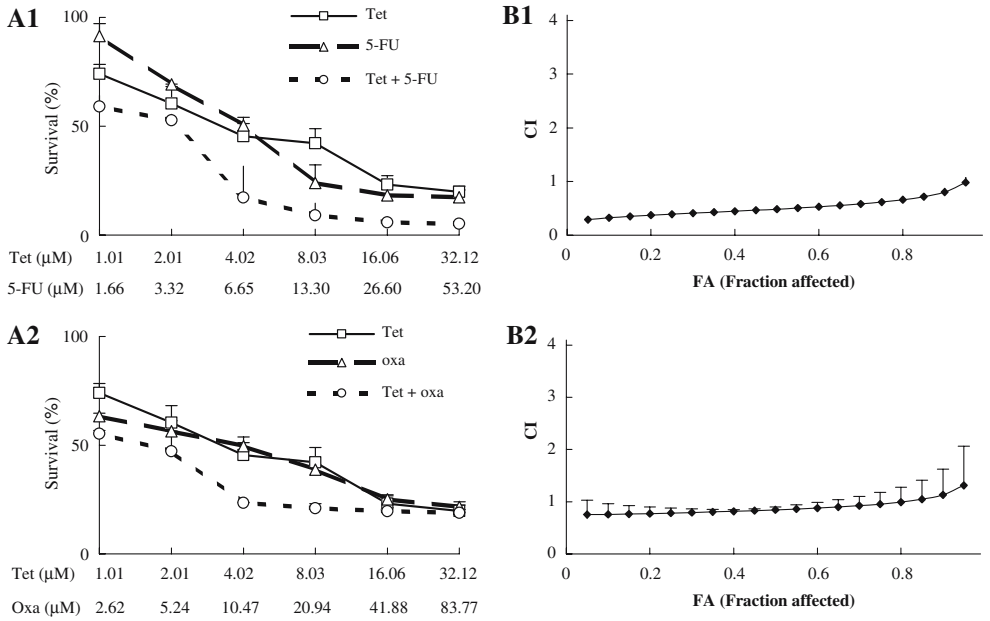

Fig. 2 Analysis of synergy between tetrandrine and 5-fluorouracil/oxaliplatin for MKN-28 cells. **a** dose–response curve of tetrandrine and chemotherapeutic agents for MKN-28 cells. Data point, means of at least three independent experiment; *bars*, SD; **b** CI values at different level of growth inhibition effect (FA). Data point, means of at least three independent experiment; *bars*, SD. **a1**, **b1** tetrandrine plus 5-fluorouracil; **a2**, **b2** tetrandrine plus oxaliplatin

Table 2 Summary of CI values at 20, 50 and 80% fraction affected

Regimen		CI at fraction affected (mean ± SD)		
		20%	50%	80%
BGC-823	Tet + Doc	0.45 ± 0.20	0.71 ± 0.31	1.77 ± 0.83
	Tet + 5-FU	10.13 ± 2.60	0.93 ± 0.06	0.35 ± 0.09
	Tet + Oxa	0.55 ± 0.34	0.65 ± 0.25	0.87 ± 0.19
MKN-28	Tet + 5-FU	0.37 ± 0.03	0.48 ± 0.04	0.66 ± 0.05
	Tet + Oxa	0.77 ± 0.12	0.84 ± 0.05	0.99 ± 0.28

effect. Some of CI values were below 1 across the almost entire dose inhibition range. Moreover, the CI values obtained for MKN-28 cells indicated a more pronounced synergistic effect for the drug combination.

We also evaluated the effects of sequential drug exposure, in which either tetrandrine or chemotherapeutic agents was administered alone for 24 h before administration of the second drug. The treatment schedule with tetrandrine preceding chemotherapeutic agents showed a similar synergistic growth inhibitory effect to the simultaneous treatment regimen, while the synergism were not observed in another occasion (sequential exposure to chemotherapeutic agents followed by tetrandrine). For example, the CI values for MKN-28 cells at 50% fraction affected were 0.48 ± 0.04, 0.73 ± 0.003 and 1.47 ± 0.45, respectively for simultaneous treatment, tetrandrine preceding 5-fluorouracil and 5-fluorouracil preceding tetrandrine. The results suggested that the simultaneous treatment and administration of tetrandrine followed by chemotherapeutic agents treatments were better than the reverse sequence treatment.

Apoptosis effects mediated by tetrandrine and chemotherapeutic agents

To test the hypothesis that tetrandrine plus chemotherapeutic agents would increase cell death by inducing apoptosis, flow cytometric analysis was performed to better understand the apoptosis effects of combining tetrandrine and chemotherapeutic agents. The double staining with both Annexin-V-FITC and PI was employed to distinguish the apoptotic cells from others [14].

BGC-823 and MKN-28 cell lines were treated with tetrandrine and anticancer drugs singly and in combination. The doses of agents chosen were close to their respective 75% inhibitory concentrations (IC_{75}). The percentage of the early apoptotic cells produced by the individual chemotherapeutic agents was significant increased by the presence of tetrandrine, indicating that the simultaneous treatment of tetrandrine and anticancer drugs induced apoptotic in a synergistic manner (Fig. 3). For example, the percentage of early apoptosis MKN-28 cells induced by tetrandrine, 5-fluorouracil and oxaliplatin were 30.29, 7.90 and 8.88%,

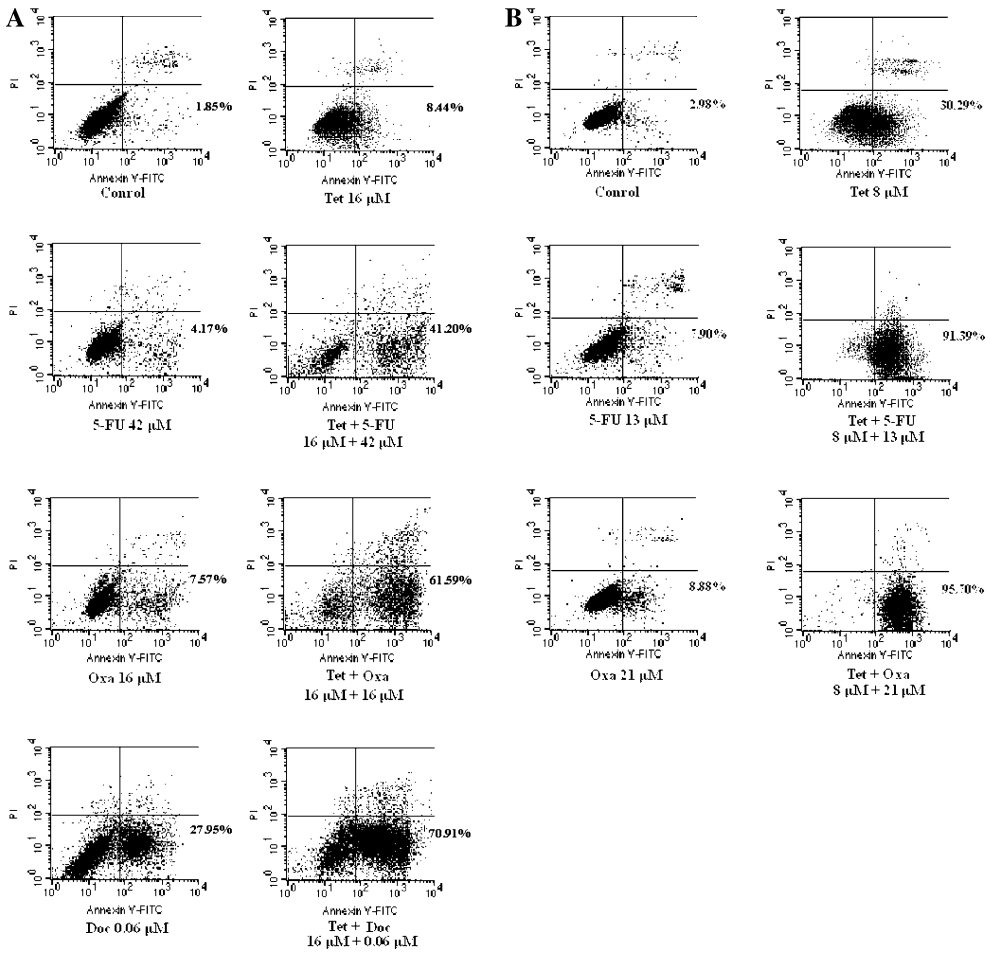

Fig. 3 Annexin-V and PI double staining for apoptosis on BGC-823 (**a**) and MKN-28 (**b**) cells. Early apoptotic cells were defined as Annexin V-positive, PI-negative cells

respectively, while the percentage of them induced by drug combination were 91.39 and 95.70% for tetrandrine plus 5-fluorouracil and tetrandrine plus oxaliplatin, respectively. Moreover, the apoptosis effect obtained for BGC-823 cells indicated a weaker synergistic effect for the drug combination than what for MKN-28 cells, which is similar to the results observed in the cytotoxicity effects.

Tetrandrine influence mRNA expression of chemotherapeutic agents-associated genes

To extend the observation made in two gastric cancer cell lines that the cytotoxicity of chemotherapeutic agents was potentiated in the presence of tetrandrine, we hypothesized that tetrandrine might affect the expression of the investigated chemotherapeutic agents-associated genes, i.e., excision repair cross-complementing (ERCC1), thymidylate synthase (TS), class III β-tubulin (β-tubulin III) and tau, in gastric cancers, influencing sensitivity to those drugs. After incubation with tetrandrine, mRNA expressions of these genes in gastric cancer BGC-823 cells were assessed by quantitative RT-PCR. As shown in Fig. 4, a significant change was observed in the expression of those genes. Most prominently, ERCC1, TS and β-tubulin III mRNA levels were markedly suppressed at 0.29-, 0.12- and 0.60-fold respectively by the presentation of tetrandrine. Meanwhile, the mRNA expression of tau was slightly inhibited. Although the mechanism of the interaction of tetrandrine and chemotherapeutic drugs was not clear enough, it is notable that tetrandrine could influence the expression of chemotherapeutic agent related genes, which might increase the sensitivity to these agents.

Discussion

Currently, the treatment of cancer with chemotherapeutic agents has two major problems: time-dependent development of tumor resistance to chemotherapy and

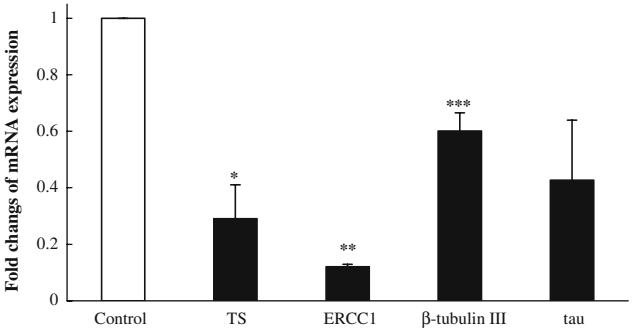

Fig. 4 Tetrandrine suppressed the mRNA expressions of chemotherapeutic agents-associated genes for BGC-823 cells. Fold changes: relative gene expression after treatment of tetrandrine at its IC_{50} for 72 h to control. $^{*}P = 0.009$; $^{**}P = 0.005$; $^{***}P = 0.009$

nonspecific toxicity toward normal cells. Many plant-derived compounds have been studied intently for their potential chemo-preventive properties and are pharmacological safe. Recent research has suggested that these compounds, such as genistein, curcumin, emodin etc., might be used to sensitize tumor cells to chemotherapeutic agents by inhibiting pathway that leads to treatment resistance [3].

The data presented here demonstrate a synergistic interaction between tetrandrine and series of chemotherapeutic agents for two gastric cancer cell lines. The potentiating action of tetrandrine in combination with other drugs was also found in the chloroquine [15], doxorubicin and vincristine [16] in vitro. Overall, MKN-28 cells were more sensitive to the tetrandrine, 5-fluorouracil and Oxaliplatin as well as the combinations. The synergy with these drug combinations is also shown in BGC-823 cells, eventhough, in some extent of concentrations, it was demonstrated as an antagonism interaction.

Several previous studies have demonstrated that the effects of combinations of antitumor drugs can vary depending on the tumor cell line tested [17, 18]. In this study, a synergy interaction of the combinations of tetrandrine and chemotherapeutic agents was observed in two different differentiated gastric cancer cell lines. Similar experiments could be carried out in other cell lines to evaluate further the potential of such combinations against different types of tumor cells.

We have examined the apoptosis effects of tetrandrine and chemotherapeutic agents on two gastric cancer cell lines, both singly and in combination, to determine whether the synergistic antiproliferative effects of the drugs observed might be because of a synergistic effect on apoptosis.Results of apoptosis analysis indicated that tetrandrine appears to be a promising candidate for combining with these three anticancer drugs, especially 5-fluorouracil and oxaliplatin. These drugs induced DNA damage directly or indirectly [19] and induced cell death predominatly via a p53-dependent pathway [20]; several recent reports have suggested that tubulin-interacting agents such as docetaxel induce cell death predominantly via another pathway [21, 22], such as caspase-dependent pathway. On other hands, several researches found out tetrandrine induced apoptosis of cancer cells via caspase-dependent pathway [8, 23]. Therefore, the pathway induced by tetrandrine leading to apoptosis would be complementary to that induced by these chemotherapeutic agents. This would at least in part explain the synergy effects of tetrandrine in combination of these three drugs. Nevertheless, this hypothesis needs to be explored by specific studies on the cell cycles and apoptosis.

The quantitative determination of synergism or antagonism by itself does not provide information about how and why synergism or antagonism occurs. We have considered how tetrandrine and these three anticancer agents might act together at a cellular level. Since several drug associated genes have been shown to be prognostic markers of 5-fluorouracil, oxaliplatin and docetaxel, we detected the influence on those genes by the presence of tetrandrine.

TS is a target enzyme for the antimetabolite 5-fluorouracil and plays a very important part in the efficacy of 5-fluorouracil. High TS expression level is reported to contribute to a resistance to 5-fluorouracil and poor clinical outcome [24, 25]. Although Some studies have shown opposite results using cancer cell lines or colorectal cancer tissue [26, 27], a meta-analysis [28] demonstrated that TS expression level was considered to be one of the most important marker to 5-fluorouracil response.

The cytotoxicity effects of the platinum agents are principally attributable to the formation of intrastrand adducts leading to the DNA damage [29]. ERCC1 is a critical element of DNA repair pathway. High ERCC1 expression is associated with resistance to platinum-containing therapy in human ovarian [30], lung [31] and gastric [32] cancer. ERCC1 has been shown to be an independent prognostic marker of platinum-based chemotherapy [33].

β-tubulin III and tau were microtuble-associated genes that were both involved in taxanes resistance. It was reported that β-tubulin III is remarkably overexpressed in paclitaxel-resistance tumors, and most importantly, the differences noticed at the mRNA level are actually translated at the protein level [34]. So, β-tubulin III overexpression is a prominent mechanism of paclitaxel resistance. Moreover, low tau expression

represents a unique molecular mechanism of hypersensitivity to paclitaxel. Inhibition of tau function could be explored as a potential therapeutic strategy to increase the anticancer activity of paclitaxel [35].

In the present study, we found that TS, ERCC1, β-tubulin III and tau were downregulated by tetrandrine: this might be a potential factor to sensitize 5-fluorouracil, oxaliplatin and docetaxel. It might be a potential reason to explain why synergistic effects of simultaneous treatment and administration of tetrandrine preceding chemotherapeutic agents differ from the reverse schedule. Therefore, further studies of how tetrandrine influence these gene expressions should be carried out.

In conclusion, this study has first demonstrated that tetrandrine might have a promising role in enhancing the efficacy of 5-fluorouracil, oxaliplatin and docetaxel in the treatment of human gastric cancer. Its potential mechanism would be their synergistic effects on apoptosis and the downregulation of chemotherapeutic agent-associated genes. Further, preclinical and clinical studies should provide additional insights and assist in determining the optimal dose and schedule for this combination in clinical use. It is clear, however, that tetrandrine has considerable promise as an adjuvant to chemotherapy.

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References

1. Sastre J, Garcia-Saenz JA, Diaz-Rubio E (2006) Chemotherapy for gastric cancer. World J Gastroenterol 12:204–213
2. Ajani JA (2005) Evolving chemotherapy for advanced gastric cancer. Oncologist 10:49–58
3. Garg AK, Buchholz TA, Aggarwal BB (2005) Chemosensitization and radiosensitization of tumors by plant polyphenols. Antioxid Redox Signal 7:1630–1647
4. Kuroda H, Nakazawa S, Katagiri K, Shiratori O, Kozuka M (1976) Antitumor effect of bisbenzylisoquinoline alkaloids. Chem Pharm Bull (Tokyo) 24:2413–2420
5. Choi HS, Kim HS, Min KR, Kim Y, Lim HK, Chang YK, Chung MW (2000) Anti-inflammatory effects of fangchinoline and tetrandrine. J Ethnopharmacol 69:173–179
6. Hui SC, Chan TY, Chen YY (1996) Tetrandrine inhibits lipid peroxidation but lacks reactivity towards superoxide anion and hydrogen peroxide. Pharmacol Toxicol 78:200–201
7. Ma JY, Barger MW, Hubbs AF, Castranova V, Weber SL, Ma JK (1999) Use of tetrandrine to differentiate between mechanisms involved in silica-versus bleomycin-induced fibrosis. J Toxicol Environ Health A 57:247–266
8. Oh SH, Lee BH (2003) Induction of apoptosis in human hepatoblastoma cells by tetrandrine via caspase-dependent Bid cleavage and cytochrome c release. Biochem Pharmacol 66:725–731
9. Choi SU, Park SH, Kim KH, Choi EJ, Kim S, Park WK, Zhang YH, Kim HS, Jung NP, Lee CO (1998) The bisbenzylisoquinoline alkaloids, tetrandine and fangchinoline, enhance the cytotoxicity of multidrug resistance-related drugs via modulation of P-glycoprotein. Anticancer Drugs 9:255–61
10. Chou TC, Talalay P (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 22:27–55
11. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
12. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402–408
13. Barret JM, Etievant C, Hill BT (2000) In vitro synergistic effects of vinflunine, a novel fluorinated vinca alkaloid, in combination with other anticancer drugs. Cancer Chemother Pharmacol 45:471–476
14. Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C (1995) A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods 184:39–51
15. Ye ZG, Van Dyke K, Castranova V (1989) The potentiating action of tetrandrine in combination with chloroquine or qinghaosu against chloroquine-sensitive and resistant falciparum malaria. Biochem Biophys Res Commun 165:758–765
16. Sun AX, Ye ZG, Li CY, Xue BY, Li LF, Cao XF, Yang Q, Dai BQ (1999) Synergistic anticancer effects of tetrandrine combined with doxorubicin or vincristine in vitro. Zhongguo Yao Li Xue Bao 20:69–73
17. Kano Y, Akutsu M, Tsunoda S, Mori K, Suzuki K, Adachi KI (1998) In vitro schedule-dependent interaction between paclitaxel and SN-38 (the active metabolite of irinotecan) in human carcinoma cell lines. Cancer Chemother Pharmacol 42:91–98
18. Perez EA, Buckwalter CA (1998) Sequence-dependent cytotoxicity of etoposide and paclitaxel in human breast and lung cancer cell lines. Cancer Chemother Pharmacol 41:448–452
19. Kaye SB (1998) New antimetabolites in cancer chemotherapy and their clinical impact. Br J Cancer 78:1–7
20. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW (1991) Participation of p53 protein in the cellular response to DNA damage. Cancer Res 51:6304–6311
21. Iwadate Y, Tagawa M, Fujimoto S, Hirose M, Namba H, Sueyoshi K, Sakiyama S, Yamaura A (1998) Mutation of the p53 gene in human astrocytic tumours correlates with increased resistance to DNA-damaging agents but not to anti-microtubule anti-cancer agents. Br J Cancer 77:547–551
22. Schimming R, Mason KA, Hunter N, Weil M, Kishi K, Milas L (1999) Lack of correlation between mitotic arrest or apoptosis and antitumor effect of docetaxel. Cancer Chemother Pharmacol 43:165–172
23. Meng LH, Zhang H, Hayward L, Takemura H, Shao RG, Pommier Y (2004) Tetrandrine induces early G1 arrest in human colon carcinoma cells by down-regulating the activity and inducing the degradation of G1-S-specific cyclin-dependent kinases and by inducing p53 and p21Cip1. Cancer Res 64:9086–9092
24. Salonga D, Danenberg KD, Johnson M, Metzger R, Groshen S, Tsao-Wei DD, Lenz HJ, Leichman CG, Leichman L, Diasio RB, Danenberg PV (2000) Colorectal tumors responding to 5-fluorouracil have low gene expression levels of dihydropyrimidine dehydrogenase, thymidylate synthase, and thymidine phosphorylase. Clin Cancer Res 6:1322–1327

25. Aschele C, Lonardi S, Monfardini S (2002) Thymidylate Synthase expression as a predictor of clinical response to fluoropyrimidine-based chemotherapy in advanced colorectal cancer. Cancer Treat Rev 28:27–47
26. Grem JL, Danenberg KD, Behan K, Parr A, Young L, Danenberg PV, Nguyen D, Drake J, Monks A, Allegra CJ (2001) Thymidine kinase, thymidylate synthase, and dihydropyrimidine dehydrogenase profiles of cell lines of the National Cancer Institute's Anticancer Drug Screen. Clin Cancer Res 7:999–1009
27. Nita ME, Tominaga O, Nagawa H, Tsuruo T, Muto T (1998) Dihydropyrimidine dehydrogenase but not thymidylate synthase expression is associated with resistance to 5-fluorouracil in colorectal cancer. Hepatogastroenterology 45:2117–2122
28. Popat S, Matakidou A, Houlston RS (2004) Thymidylate synthase expression and prognosis in colorectal cancer: a systematic review and meta-analysis. J Clin Oncol 22:529–536
29. Fehrenbach A, Nusse N, Nayudu PL (1998) Patterns of growth, oestradiol and progesterone released by in vitro cultured mouse ovarian follicles indicate consecutive selective events during follicle development. J Reprod Fertil 113:287–297
30. Selvakumaran M, Pisarcik DA, Bao R, Yeung AT, Hamilton TC (2003) Enhanced cisplatin cytotoxicity by disturbing the nucleotide excision repair pathway in ovarian cancer cell lines. Cancer Res 63:1311–1316
31. Rosell R, Cecere F, Santarpia M, Reguart N, Taron M (2006) Predicting the outcome of chemotherapy for lung cancer. Curr Opin Pharmacol 6:323–331
32. Metzger R, Leichman CG, Danenberg KD, Danenberg PV, Lenz HJ, Hayashi K, Groshen S, Salonga D, Cohen H, Laine L, Crookes P, Silberman H, Baranda J, Konda B, Leichman L (1998) ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. J Clin Oncol 16:309–316
33. Sarries C, Haura EB, Roig B, Taron M, Abad A, Scagliotti G, Rosell R (2002) Pharmacogenomic strategies for developing customized chemotherapy in non-small cell lung cancer. Pharmacogenomics 3:763–780
34. Mozzetti S, Ferlini C, Concolino P, Filippetti F, Raspaglio G, Prislei S, Gallo D, Martinelli E, Ranelletti FO, Ferrandina G, Scambia G (2005) Class III beta-tubulin overexpression is a prominent mechanism of paclitaxel resistance in ovarian cancer patients. Clin Cancer Res 11:298–305
35. Rouzier R, Rajan R, Wagner P, Hess KR, Gold DL, Stec J, Ayers M, Ross JS, Zhang P, Buchholz TA, Kuerer H, Green M, Arun B, Hortobagyi GN, Symmans WF, Pusztai L (2005) Microtubule-associated protein tau: a marker of paclitaxel sensitivity in breast cancer. Proc Natl Acad Sci USA 102:8315–8320