Enhanced sensitivity of human ovarian carcinoma cell lines A2780 and A2780/CP to the combination of cisplatin and synthetic isothiocyanate ethyl 4-isothiocyanatobutanoate*

J. BODO, J. CHOVANCOVA, L. HUNAKOVA, J. SEDLAK

Laboratory of Tumor Immunology, e-mail: exonbodo@savba.sk, Cancer Research Institute, 83391 Bratislava, Slovak Republic

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Naturally occurring and synthetic isothiocyanates (ITCs) are known as chemopreventive agents. The present study shows a new synthetic ITC derivate ethyl 4-isothiocyanatobutanoate (E-4IB) as an effective modulator of cellular proliferation and inducer of apoptosis with potential utility as an anticancer drug, as well as a sensitizer to routinely used chemotherapeutic agent cisplatin (cis-Pt).

Evaluation of the growth inhibitory effects of E-4IB in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant variant A2780/CP using MTT-test and its apoptosis-inducing properties by flow cytometry was performed.

Effect of E-4IB was assessed both alone and in paired combination with cisplatin. Combination index (CI) values from Calcusyn software were used to characterize the interactions as synergistic, additive, or antagonistic. Significant synergistic effect in growth inhibition of E-4IB (0.5–5 μM) with cis-Pt (2.5–10 μM) on A2780 parental cell line (CI from 0.39 to 0.75) was also observed on A2780/CP resistant subline, although to a lesser extent (CI from 0.43 to 0.86) for cis-Pt concentrations 5–25 μM and the same concentrations of E-4IB. Synergy in growth inhibition correlated with the potential of E-4IB to stimulate apoptosis induced by cis-Pt (from 9.5% to 24.7% at 24 hours) while E-4IB alone induced 3.6% of apoptotic cells in A2780 cell line.

We conclude that E-4IB may be worth of further studies assessing its value in the ovarian carcinoma treatment, in combination with the other chemotherapeutic agents.

Key words: ovarian carcinoma, isothiocyanate, cisplatin, growth inhibition, apoptosis

Carcinogenesis is a multistep process of malignant transformation progressing towards uncontrolled proliferation, invasion and metastasis. Cancer chemoprevention, as a new approach in the management of cancer development, uses non-cytotoxic drugs and natural agents to reverse, suppress or prevent this progression to invasive disease [5].

ITCs, naturally occurring food components, inhibitors of phase I enzymes and inducers of phase II enzymes, are known as chemopreventive agents. Their precursors are constituents of cruciferous vegetables (such as cabbage, cauliflower, Brussels sprouts, broccoli), which consumption is associated with a reduced incidence of cancer [13]. They can also induce apoptosis and mediate growth inhibition of human cancer cells *in vitro* [9, 18]. ITCs, by their direct interaction with GSH and forming dithiocarbamate conjugate [R-NH-C(=S)-SG], can cause depletion of the intracellular GSH level and subsequent oxidative stress [24]. Activation of three MAP kinases (JNK, ERK and p38 kinase) was described after ITCs treatment in tumor cells [11, 22, 23].

Based on *in vitro* [7] and *in vivo* [17] biological activities of synthetic ITCs tested, E-4IB, formally a derivate of gamma-aminobutyric acid (GABA), was chosen for further studies as the most potent cytotoxic compound [17].

Platinum compounds are important agents used in treatment of many cancers including ovarian carcinomas, where they compose basic components of the first-line chemotherapy. However, frequent development of resistance to platinum-based chemotherapy limits its use and stimulates the

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search for combination therapy aimed at circumvention or decreasing of platinum resistance [1, 2].

Cellular resistance to platinum can arise by multiple mechanisms related to GSH-based detoxification and metallothionein content, MRP2 or ATP7B transporters overexpression, an increase in DNA repair capacity, but also because of aberrant properties of signal transduction pathways involved in cell cycle control and apoptosis (for example p53 alterations, Bcl-2 expression, ERK activation) [21].

In this study we show combined synergistic effect of synthetic ITC E-4IB and platinum compound cisplatin treatment in human ovarian adenocarcinoma cell line A2780 and its cisplatin-resistant subline A2780/CP in growth inhibition and induction of apoptosis.

Material and methods

Reagents. Ethyl 4-isothiocyanatobutanoate (E-4IB) was synthesized as described [6]. Dimethyl sulfoxide (DMSO), RNA-se A, fluorescein diacetate (FDA), propidium iodide (PI) and 3-[4. 5-dimethylthiazol-2-yl]-2.5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO). Cisplatin (cis-Pt) was acquired from LACHEMA (Czech Republic).

Cell culture and treatment. The human ovarian carcinoma cell line A2780 and its cisplatin-resistant variant A2780/CP were routinely cultured in RPMI640 medium supplemented with 10% fetal calf serum, 2 mM L-Glutamine, 100 μg/ml penicillin and 50 μg/ml streptomycin in humified air atmosphere with 5% CO_2 at 37 °C. The cultures were passaged twice a week after reaching cell density of 0.8–1.0 x 10^6 cells/ml. Cells were plated at 3–6 x 10^4 cells/cm^2 density on day before treatment. Cells were exposed to various concentrations of E-4IB or cis-Pt for the respective time indicated. In combination, E-4IB was always added three hours before cis-Pt treatment (based on our previous experiments showing that this time is sufficient to activate E-4IB inducible signaling pathways). Stock solution of E-4IB was originally dissolved in DMSO, and an equal volume of DMSO (final concentration <0.02%) was added to the cells.

Flow cytometric analysis of apoptosis. Apoptotic cell enumeration was done according to the amount of cells with the cell membrane impermeable for PI with low amount of fluorescein, or cells with sub-G_1 amount of DNA after detergent permeabilization. As for FDA/PI staining [3], both cell lines were collected as described above. Pooled cells were washed twice with cold PBS. Approximately 5 x 10^5 cells were resuspended in 400 μl of PBS/0.2% BSA containing 10 nM of FDA (from a 5 mM stock in DMSO) for 30 min at room temperature. Then cells were cooled and 4 μl of PI (1 mg/ml) was added. Finally, cells were analyzed using a Coulter Epics Altra flow cytometer.

Cell cycle analysis. This determination was based on the measurement of the DNA content of nuclei labeled with propidium iodide. For the flow cytometry analyses of the DNA cell cycle profile, cells were washed twice with cold PBS and resuspended in 0.05% Triton X-100 and 15 μl RNA-se A (10 mg/ml) for 20 min at 37 °C. Then cells were cooled and incubated on ice for at least 5 min before propidium iodide (50 μg/ml) was added. Finally, after 15 min the stained cells were analyzed using a Coulter Epics Altra flow cytometer.

Flow cytometry measurements and data analysis. Coulter Epics Altra flow cytometer was equipped with 488 nm excitation laser and fluorescence emission was measured using bandpass filter set 525, 575, 610, 675 nm with respective photomultipliers FL1-FL4 required for fluorochrome used as follows: PI (FL2), FDA and PI (FL1, FL2), cell cycle (log FL2 – sub G_1, lin FL3 – DNA cell cycle histogram, lin/peak FL4 – discrimination of doublets). Forward/side light scatter characteristic was used to exclude the cell debris from the analysis. For each analysis, 1 x 10^4 cells were required. Data were analyzed with WinMDI version 2.8 software (J. Trotter, Scripps Research Institute, La Jolla, CA). The cell cycle calculations were performed with MULTI-CYCLE Software (Phoenix Flow System).

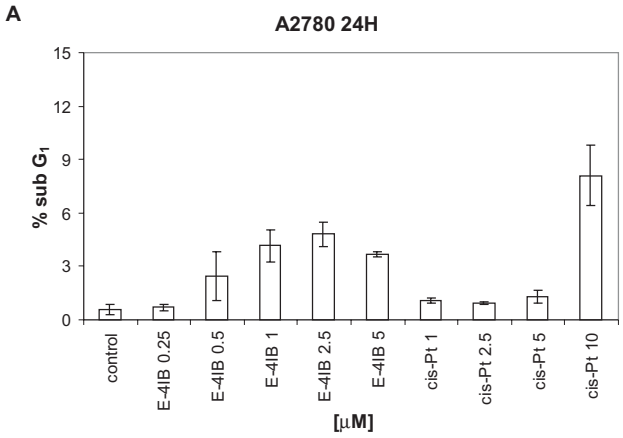
Cytotoxicity assay. Effect of E-4IB, cis-Pt or their combination on survival of cells was determined by MTT assay [14]. Cells were seeded at (1–2) x 10^3 cell density in 96-well culture plates. Each dose of tested compounds (added it the volume of 50 μl) was tested in triplicate, and cytotoxicity curve was constructed from at least seven different concentrations. After 72 h, the cells were incubated with 50 μl of MTT (1 mg/ml) and left in the dark at 37 °C for an additional 4 h. Thereafter, medium was removed, the formazan crystals were dissolved in 200 μl of DMSO, and the absorbance was measured at 540 nm and 690 nm in Microplate reader (Dynatech Lab Inc., Chantilly, VA, USA). The concentration of drug that inhibited cell survival to 50% (IC_{50}) and combination index (CI) were determined by Calcusyn software (version 1.1, Biosoft)

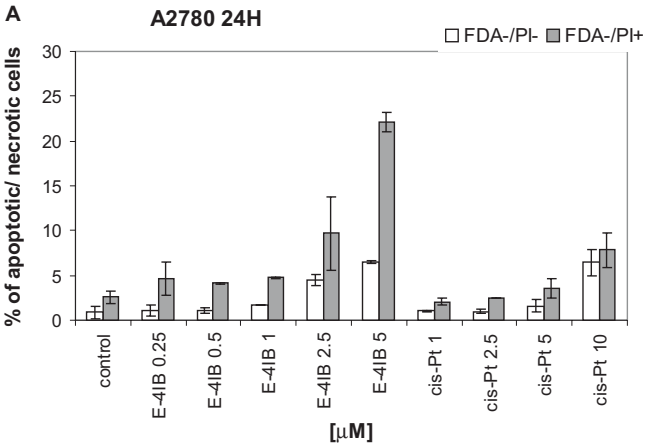
Results

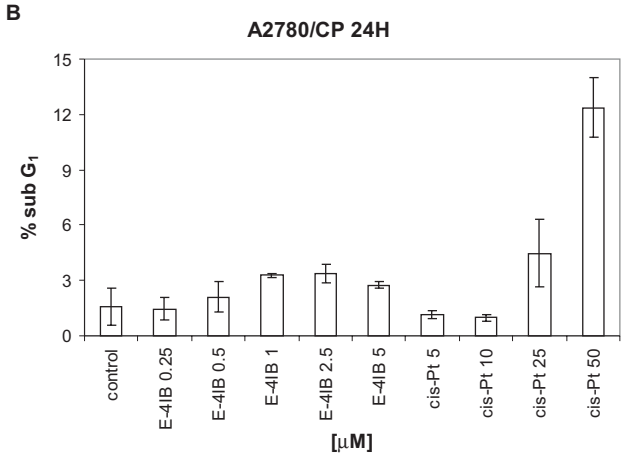
E-4IB and cis-Pt induce apoptosis and necrosis. To get insight into the effect of E-4IB we determined the contribution of apoptosis to observed cell death. Two independent flow cytometry methods were used for evaluation of apoptosis induced by E-4IB.

The presence of apoptotic cells determined by the analysis of DNA fragmentation (sub-G_1 population) is shown in Figure 1. This figure illustrates the concentration-dependent effect of E-4IB and cis-Pt treatment on tested cell lines. Single compound-treated cells have a slight increase of cell population with sub-G_1 DNA content in A2780 (approximately 5% by E-4IB or 7% by cis-Pt – 10 μM) and in A2780/CP cells (2% by E-4IB or 11% by cis-Pt – 50 μM) after 24 h treatment.

To confirm the apoptotic process, a second technique – FDA/PI staining, was used. Figure 2 depicts the percentage of







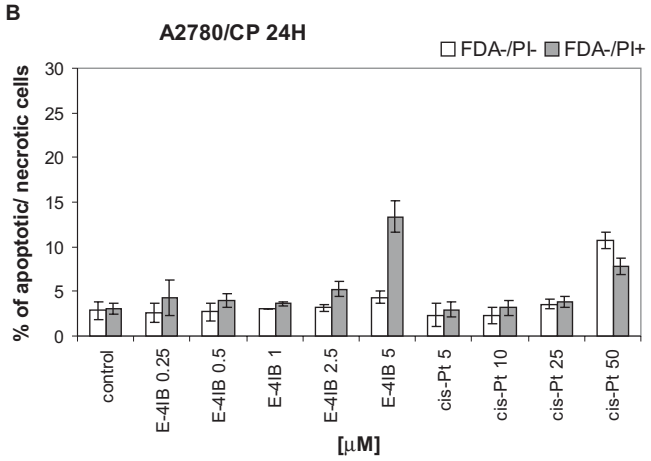


Figure 1. Effect of E-4IB or cis-Pt on sub-G_1 population in A2780 (A), A2780/CP (B) treated cell lines. The cells were exposed to DMSO (control) or 0.25–5 μM of E-4IB and 1–10 μM (A) or 5–50 μM of cis-Pt (B) for 24 h. Percentage of sub-G_1 fraction was obtained from analysis of SSC versus log FL2 dot plot using WinMDI software. The data presented are representative of at least two independent experiments. Statistical significance from the controls, *p<0.05, **p<0.01.

Figure 2. Effect of E-4IB or cis-Pt treatment on apoptosis and necrosis induction in A2780 (A) and A2780/CP (B) cells. Cells were exposed to 0.25–5 μM of E-4IB and 1–10 μM (A) or 5–50 μM of cis-Pt (B) for 24 h. The FDA stained cells were analyzed using a Coulter Epics Altra flow cytometer. Percentage of apoptotic (FDA^-/PI^-) and late apoptotic/necrotic (FDA^-/PI^+) cells was illustrated. The data presented are representative of two independent experiments. Statistical significance from the controls, *p<0.05, **p<0.01.

apoptotic (FDA^-/PI^-) and necrotic (FDA^-/PI^+) cells after 24 h of treatment. Results demonstrate that E-4IB and cis-Pt induced significant increase of percentage of apoptotic cells after 24 h, in accordance with DNA analysis. Number of necrotic cells also increased in a concentration-dependent manner.

E-4IB-induced cell cycle changes. The effect of E-4IB on proliferation of cis-Pt-treated cells was evaluated by measuring the distribution of cells in the different cell cycle phases. This cell distribution was done by histogram analysis of PI stained cells. Figure 3 shows histograms of cell cycle analysis of combined E-4IB and cis-Pt treatment. E-4IB alone induced G2/M block in A2780 cells, while cis-Pt caused S-phase arrest in both cell lines, more advanced in parental cells (early S-phase). In combination, cells were exposed to E-4IB (0.5 μM) for 3 h prior to 24 h treatment of cis-Pt (10 μM). In comparison to single cis-Pt treatment decrease of proliferation in both tested cell lines was observed. We have found approximately 20% decrease of S-phase cells in A2780 and 10% decrease in A2780/CP cells.

E-4IB significantly increases cis-Pt-induced apoptosis and necrosis. E-4IB pre-treatment induced significant increase of cells with sub-G_1 content in comparison to single cis-Pt treatment in both tested cell lines (Fig. 4). The highest accumulation of apoptotic population in A2780 cells (approximately 25%) was found by combination of E-4IB 0.5 μM and cis-Pt 10 μM (Fig. 4a). Synergistic effect in

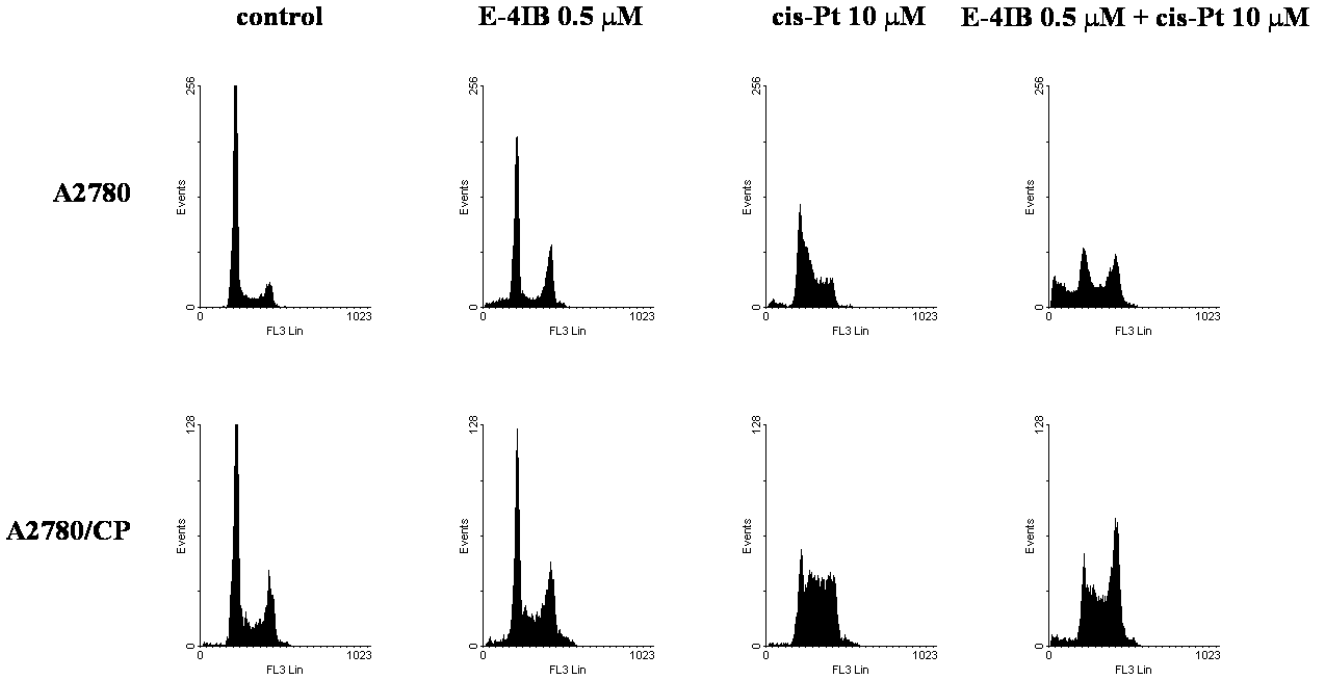


Figure 3. Flow cytometry-based cell cycle analysis of E-4IB, cis-Pt and their combination in A2780 (A) and A2780/CP (B) cells. The cells were exposed to DMSO (control) or E-4IB (0.5 μM) for 3 h and then to cis-Pt (10 μM) without washing for 24 h. The cells were collected, detergent permeabilized and stained with 50 μM/ml concentration of PI in the presence of RNA-se A. The distribution of cells in G_0/G_1, S and G_2/M phase was analyzed with Multi-cycle software.

A2780/CP cells was lower but statistically significant ($p<0.05$) (Fig. 4b).

FDA analysis showed (Fig. 5) that E-4IB amplified cis-Pt-induced apoptosis in both treated cell lines, which correlated with DNA analysis. After combination treatment, the proportion of necrotic (propidium iodide positive) cells, was 17% in A2780 cells, whereas in A2780/CP it was only 7%. Necrotic cell population was increased in a concentration-dependent manner.

Cytotoxicity of E-4IB. To assess the effect of E-4IB, cells were treated with different concentrations of E-4IB and cis-Pt or both (Fig. 6). Combination index (CI) values (Calcusyn 1.1, Biosoft, 1996) were used to characterize the interactions as synergistic (<1), additive (=1), or antagonistic (>1). Significant synergistic effect of E-4IB (0.5–5 μM) with cis-Pt (2.5–10 μM) on A2780 parental cell line (CI from 0.39 to 0.75) was observed also on A2780/CP resistant subline, although to a lesser extent (CI from 0.43 to 0.86) for cis-Pt concentrations 5–25 μM. Values of IC_{50} for E-4IB and cis-Pt alone were determined from cell survival plots and were 1.7±0.6 μM in A2780 and 1.3±0.4 μM in A2780/CP for E-4IB, and 5.0±0.6 μM in A2780 and 13±3.1 μM in A2780/CP for cis-Pt after 72 hour treatment. Dose reduction effects characterized by dose reduction index (DRI), which determines how many fold increase of each drug concentration (if used alone) is required for the same effect as is provided by the corresponding synergistic combination, have values as follows: 4.4 for E-4IB and 12.5 for cis-Pt in A2780 cells and 5.5 for E-4IB and 2.7 for cis-Pt in A2780/CP cells at IC_{50} values combination line.

Discussion

Platinum compounds are routinely used in cancer treatment, but acquired resistance often diminishes the efficacy of these drugs [21]. The evaluation of novel agents for the treatment of ovarian cancer is important, since even after initial response to the conventional chemotherapy relapse is common and the recurrent disease may be resistant to standard agents [12]. Accumulated evidence supports a role for altered signaling in mediating resistance. One of the ways to enhance the efficacy of cisplatin-treatment in sensitive cancers and to overcome drug resistance in unresponsive tumors, is to target involved signaling pathways with specific compounds in combination with cisplatin [20].

Previous studies [7, 8] have demonstrated that E-4IB can inhibit the growth of sarcoma and adenocarcinoma cells *in vi-*

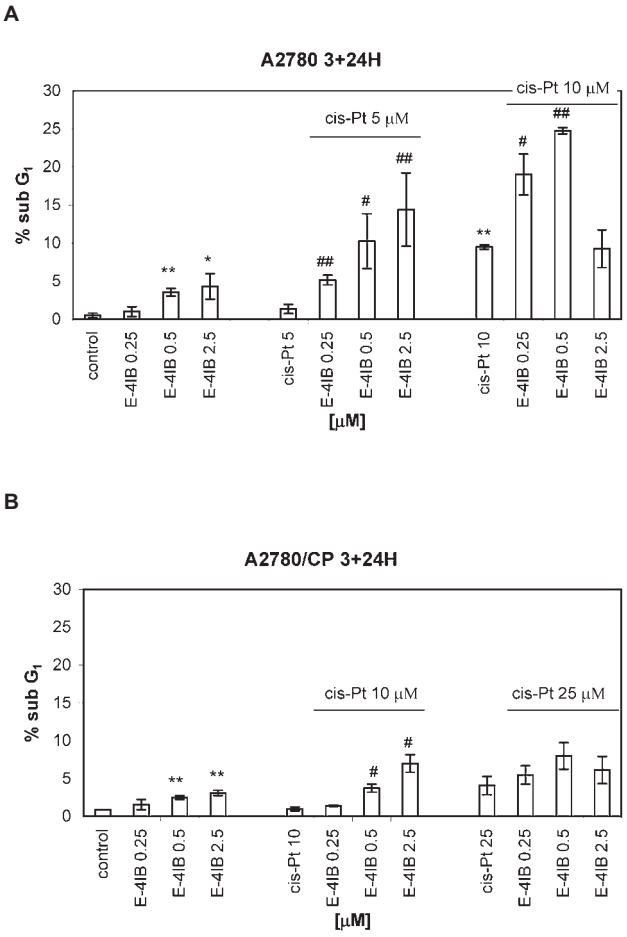


Figure 4. Combination effect of E-4IB and cis-Pt on generation of sub-G_1 population in A2780 (A), and A2780/CP (B) cell lines. The cells were exposed to DMSO (control) or different concentration of E-4IB (0.25, 0.5 and 2.5 μM) for 3 h and then to cis-Pt – 5 or 10 μM (A) and 10 or 25 μM (B) without washing for 24 h. Percentage of sub-G_1 fraction was obtained from analysis of SSC versus log FL2 dot plot using WinMDI software. The results shown are representative of at least three independent experiments. Statistical significant from the controls, *p, **p and from corresponding cis-Pt-treated samples, #p<0.05, ##p<0.01.

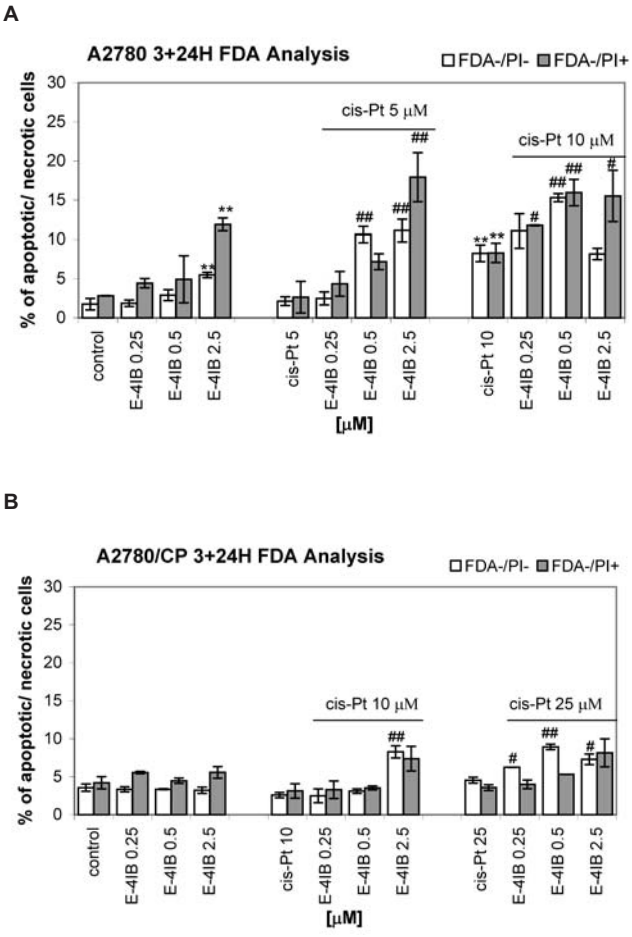


Figure 5. Combination effect of E-4IB and cis-Pt treatment on apoptosis and necrosis induction in A2780 (A) and A2780/CP (B) cells. Cells were exposed to 0.25, 0.5 and 2.5 μM of E-4IB for 3 h prior to treatment of cis-Pt – 5 or 10 μM (A) and 10 or 25 μM (B). The cells were analyzed using a coulter Epics Altra flow cytometer. Percentage of apoptotic (FDA^-/PI^-) and late apoptotic/necrotic (FDA^-/PI^+) cells was illustrated. The data presented are representative of three independent experiments. Statistical significance from the controls, *p<0.05, **p<0.01 and from corresponding cis-Pt-treated samples, #p<0.05, ##p<0.01.

tro and *in vivo* and it was illustrated that E-4IB is not immunotoxic [19]. The antitumor activities of E-4IB have been also evaluated in rats implanted with B77-RF cells [7]. However, there are no reports about E-4IB growth inhibiting properties in human gynecological cancers so far.

This study presents effects of E-4IB alone or in combination with cisplatin on human ovarian carcinoma cell lines. Our present data provide evidence that E-4IB alone inhibited the cell growth of tested cell lines and E-4IB augmented the cytotoxicity of cisplatin in both A2780 and A2780/CP cells *in vitro*. In addition, the synergistic cytotoxic effect was observed in E-4IB/cisplatin-treated cell lines. In the experiments investigating these synergistic effects, the concentrations of E-4IB and cisplatin were chosen based on our data showing dose-dependent effects of E-4IB and cisplatin alone. The viability values of cells treated with E-4IB/cisplatin were lower than viability values in cisplatin-treated cells, but if the concentration of E-4IB was too low (<0.25 μM), no synergy effect was observed. The combinations of E-4IB with cisplatin also increased cell apoptosis at subcytotoxic concentrations of cisplatin.

Molecular mechanisms of the synergistic action of E-4IB have yet to be clarified. It is know that both cisplatin [10, 16] and isothiocyanates [24] induce a decrease of GSH level and thus GSH depletion may be one of common links responsible for observed synergy in the combination treatment. However,

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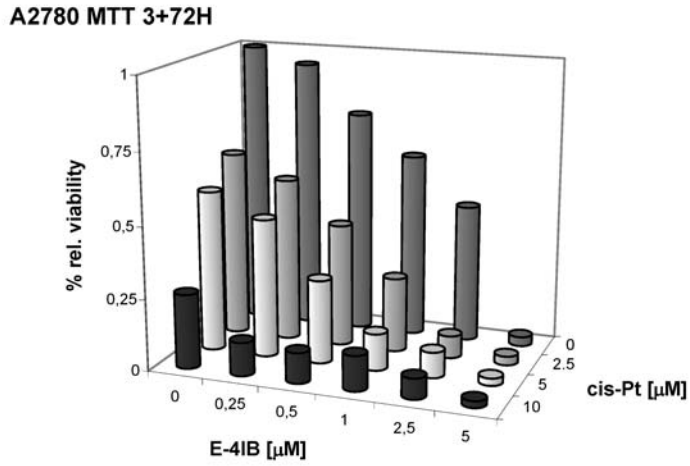
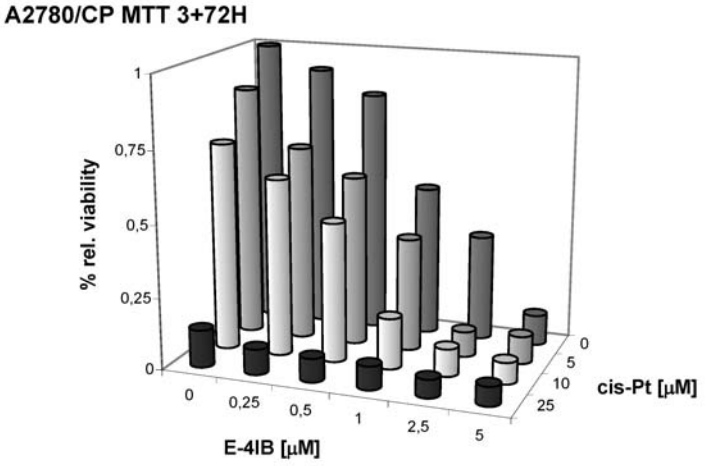


A2780	cis-Pt		
[μM]	2,5	5	10
E-4IB	Combination index		
0,25	0,95	1,21	0,44
0,5	0,75	0,72	0,46
1	0,58	0,44	0,64
2,5	0,45	0,58	0,62
5	0,42	0,39	0,45

B



A2780/CP	cis-Pt		
[μM]	5	10	25
E-4IB	Combination index		
0,25	0,97	1,04	0,54
0,5	0,82	0,86	0,57
1	0,79	0,56	0,62
2,5	0,43	0,53	0,56
5	0,54	0,49	0,60

Figure 6. Relative viability (MTT assay) of cisplatin-sensitive A2780 cells (A) and cisplatin-resistant A2780/CP cells (B) after 3 hour exposition to 0.25–5 μM E-4IB and corresponding concentration of cis-Pt. cis-Pt at concentrations 2.5–10 μM (A) and 5–25 μM (B) was added for 72 hours. Relative viability of control cells = 1.0. The tables show the calculated combination indices values. The data presented are representative of two independent experiments.

it was illustrated that although reduced concentration of intracellular GSH was linked to apoptosis [4], lowering of intracellular GSH levels by ITC observed by PULLAR et al [15] was not sufficient to sensitize the cells to apoptosis. Therefore, we can not exclude impact of other possible mechanisms, i.e. those participating in the cell cycle check-point control and apoptosis, which might lead to acquisition of cisplatin resistance possibly modulated by ITCs.

In further investigations, we will explore the changes in GSH level, the expression of intracellular protein regulation elements (such as MAP kinases) with the aim of elucidating the mechanism of the synergistic apoptotic activity in response to E-4IB and cisplatin.

In conclusion, we have shown, that E-4IB exerted cytotoxic effects and exhibited synergistic effects in combination with cisplatin in A2780 and A2780/CP cells. Since the synergism is observed over a wide range of concentrations, the dose of each reagent may be reduced, thus lowering side effect toxicity. The data suggest that the combination of chemotherapeutic drug cisplatin and E-4IB offers a promising means to overcome cisplatin-resistance and could be useful as a treatment of recurrent and resistant ovarian carcinomas. While this resulted in synergistic toxicity, additional studies are necessary to determine the optimal way of combination of cisplatin and E-4IB. These data are *in vitro* experiments with cancer cell lines and so the efficacy and specific-

ity of this combination remain to be tested in further preclinical models.

References

[1] AGARWAL R, KAYE SB. Ovarian cancer: Strategies for overcoming resistance to chemotherapy. Nat Rev Cancer 2003; 3: 502–516.

[2] BAIRD RD, KAYE SB. Drug resistance reversal – are we getting closer? Eur J Cancer 2003; 39: 2450–2461.

[3] BARTKOWIAK D, HOGNER S, BAUST H, NOTHDURFT W, ROTTINGER EM. Comparative analysis of apoptosis in HL60 detected by annexin-V and fluorescein-diacetate. Cytometry 1999; 37: 191–196.

[4] CHIBA T, TAKAHASHI S, SATO N, ISHII S, KIKUCHI K. Fas-mediated apoptosis is modulated by intracellular glutathione in human T cells. Eur J Immunol 1996; 26: 1164–1169.

[5] FIMOGNARI C, NUSSE M, BERTI F, IORI R, CANTELLI-FORTI G et al. Isothiocyanates as novel cytotoxic and cytostatic agents: molecular pathway on human transformed and non-transformed cells. Biochem Pharmacol 2004; 68: 1133–1138.

[6] FLOCH L, KUBAN J, GOGOVA A, JAKUBIK T, PRONAYOVA N. Synthesis of ethyl 4-isothiocyanatobutanoate derivatives. Chem Pap -Chem Zvesti 1997; 51: 416–420.

[7] HORAKOVA K, JANTOVA S, STREDANSKY M, NOVOTNA L, FLOCH L. Ethyl 4-isothiocyanatobutanoate - antiproliferative activity in-vitro and in-vivo. Anti-Cancer Drugs 1993; 4: 369–375.

[8] HORAKOVA K, STRAKOVA Z, JANTOVA S, MUCKOVA M, FLOCH L. Biological effects of 2-isothiocyanate carboxylic-acid esters 1. Cyto-toxic, antimicrobial and genetoxic screening amino-acid isothiocyanate derivatives. Biologia 1989; 44: 637–646.

[9] JAKUBIKOVA J, SEDLAK J, MITHEN R, BAO Y. Role of PI3K/Akt and MEK/ERK signaling pathways in sulforaphane- and erucin-induced phase II enzymes and MRP2 transcription, G(2)/M arrest and cell death in Caco-2 cells. Biochem Pharmacol 2005; 69: 1543–1552.

[10] JANSEN BAJ, BROUWER J, REEDIJK J. Glutathione induces cellular resistance against cationic dinuclear platinum anticancer drugs. J Inorg Biochem 2002; 89: 197–202.

[11] KONG ANT, YU R, HEBBAR V, CHEN C, OWUOR E et al. Signal transduction events elicited by cancer prevention compounds. Mutat Res -Fundam Mol Mech Mutagen 2001; 480: 231–241.

[12] MARKMAN M. Intraperitoneal chemotherapy is appropriate first line therapy for patients with optimally debulked ovarian cancer. Crit Rev Oncol Hematol 2001; 38: 171–175.

[13] MICHAUD DS, SPIEGELMAN D, CLINTON SK, RIMM EB, WILLETT WC et al. Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. J Natl Cancer Inst 1999; 91: 605–613.

[14] MOSMANN T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55–63.

[15] PULLAR JM, THOMSON SJ, KING MJ, TURNBULL CI, MIDWINTER RG et al. The chemopreventive agent phenethyl isothiocyanate sensitizes cells to Fas-mediated apoptosis. Carcinogenesis 2004; 25: 765–772.

[16] SAYED-AHMED MM, EISSAA MA, KENAWY SA, MOSTAFA N, CALVANI M et al. Progression of cisplatin-induced nephrotoxicity in a carnitine-depleted rat model. Chemotherapy 2004; 50: 162–170.

[17] SOVCIKOVA A, TULINSKA J, CHALUPA I, LISKOVA A, KURICOVA M et al. Immunotoxic and cancerostatic effects of ethyl-4-isothiocyanatobutanoate in female Lewis rats with implanted fibrosarcoma. Int Immunopharmacol 2002; 2: 1681–1691.

[18] SVEHLIKOVA V, WANG SR, JAKUBIKOVA J, WILLIAMSON G, MITHEN R et al. Interactions between sulforaphane and apigenin in the induction of UGT1A1 and GSTA1 in CaCo-2 cells. Carcinogenesis 2004; 25: 1629–1637.

[19] TULINSKA J, SOVCIKOVA A, LISKOVA A, KUBOVA J, HORAKOVA K. Immunotoxicity of ethyl-4-isothiocyanatobutanoate in male Wistar rats. Toxicology 2000; 145: 217–225.

[20] VASILEVSKAYA IA, RAKITINA TV, O'DWYER PJ. Quantitative effects on c-Jun N-terminal protein kinase signaling determine synergistic interaction of cisplatin and 17-allylamino-17-demethoxygeldanamycin in colon cancer cell lines. Mol Pharmacol 2004; 65: 235–243.

[21] WERNYJ RP, MORIN PJ. Molecular mechanisms of platinum resistance: still searching for the Achilles' heel. Drug Resist Update 2004; 7: 227–232.

[22] XIAO D, SRIVASTAVA SK, LEW KL, ZENG Y, HERSHBERGER P et al. Allyl isothiocyanate, a constituent of cruciferous vegetables, inhibits proliferation of human prostate cancer cells by causing G(2)/M arrest and inducing apoptosis. Carcinogenesis 2003; 24: 891–897.

[23] YU R, JIAO JJ, DUH JL, TAN TH, KONG ANT. Phenethyl isothiocyanate, a natural chemopreventive agent, activates c-Jun N-terminal kinase 1. Cancer Res 1996; 56: 2954–2959.

[24] ZHANG YS, TALALAY P. Mechanism of differential potencies of isothiocyanates as inducers of anticarcinogenic phase 2 enzymes. Cancer Res 1998; 58: 4632–4639.