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ORIGINAL ARTICLE

Combination analyses of anti-cancer drugs on human neuroendocrine tumor cell lines

Dhana E. Larsson · Sadia Hassan · Rolf Larsson · Kjell Öberg · Dan Granberg

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Abstract
Purpose There is a large need for better pharmacological treatment of neuroendocrine tumors. The aim of this study was to investigate and quantify the cytotoxic potentiating effects resulting from a combination of five substances, NSC 95397, emetine, CGP-74514A hydrochloride, Brefeldin A and sanguinarine chloride, chosen from a previous screening of 1,280 pharmacologically active agents on neuroendocrine tumor cells, with standard cytotoxic agents currently used in the treatment of neuroendocrine tumors.
Method The human pancreatic carcinoid cell line BON-1, human typical bronchial carcinoid cell line NCI-H727 and the human atypical bronchial carcinoid cell line NCI-H720 were used. Combinations between doxorubicin, etoposide, oxaliplatin, docetaxel, and each one of the five agents were studied and simultaneous exposures were explored using the median-effect method.
Results Most of the combinations of NSC-95397 and emetine with doxorubicin, etoposide, docetaxel, and oxaliplatin showed synergism, and their remaining combinations were additive. Almost all of the CGP-74514A hydrochloride interactions were additive, while brefeldin A and sanguinarine displayed less synergy but more additive and antagonistic interactions in combination with the standard drugs.
Conclusion The synergistic and additive interactions make NSC-95397, emetine, and CGP-74514A hydrochloride potential candidates for incorporation into combination chemotherapy regimens and these drugs might be the suitable candidates for further clinical studies in patients with bronchial carcinoids and pancreatic endocrine tumors.

Keywords Neuroendocrine tumors · Combination · Chemotherapy · Drug-sensitivity

D. E. Larsson (✉) · K. Öberg · D. Granberg
Department of Endocrine Oncology, Faculty of Medicine, University of Uppsala, University Hospital, 751 85 Uppsala, Sweden
e-mail: dhana.larsson@medsci.uu.se

S. Hassan · R. Larsson
Department of Medical Sciences, Division of Clinical Pharmacology, Uppsala University Hospital, Uppsala, Sweden

Introduction

Neuroendocrine tumors form a heterogeneous group of malignancies, which differ from each other in their biology, prognosis, and genetics. They mainly occur in the gastrointestinal tract, a substantial percentage, however, is found in the bronchopulmonary system [1]. Typical carcinoids and atypical carcinoids are two sub-types of lung neuroendocrine tumors, showing different clinical behavior [2, 3]. Pancreatic endocrine tumors, also known as islet cell carcinomas, are another rare type of neuroendocrine tumor [4]. Although localized carcinoids or islet cell tumors are surgically manageable, metastatic disease is present in nearly 50% of patients at the time of diagnosis [5]. The use of various chemotherapeutic agents, such as doxorubicin, 5-fluorouracil, cisplatin, carboplatin, etoposide streptozotocin, and temozolomide has led to minimal responses in the treatment of patients with lung carcinoids [6, 7] and pancreatic endocrine tumors [8, 9], mostly of short duration. The low response rates for chemotherapy and the side effects underscore the need for new therapeutic options in these neoplasms.

Modern cancer chemotherapy is mainly based on combination therapy rather than single agent treatment in order

to maximize the tumor killing effect and to limit drug-specific toxicity. Since animal and clinical studies are laborious and costly, an important aspect of early cancer drug development is the preclinical in vitro evaluation of drugs in cell lines. The median effect analysis method of Chou and Talalay is a commonly used approach for evaluating efficacy potentiation using drug combinations [10]. The method is a general equation for dose–effect relationship through mathematical induction using hundreds of enzyme kinetic models. This method allows evaluation of antagonistic, additive, and synergistic interaction of drug combinations.

In our earlier screening study of 1,280 pharmacologically active compounds using neuroendocrine tumor cell lines, 11 compounds were found to cause tumor cell death at low concentration [11]. Five of these compounds, with different mechanisms of action, were chosen for further studies aiming at evaluating potential synergistic effects when combined with four standard cytotoxic drugs already used in the clinic for treatment of neuroendocrine tumors. The five chosen compounds were NSC-95397, a selective Cdc25 dual specificity phosphatase inhibitor; emetine, a protein synthesis inhibitor and DNA interacting agent; CGP-74514A hydrochloride, a cyclin-dependent kinase-1 inhibitor; brefeldin A, the inhibitor of the protein transport from the endoplasmic reticulum to the Golgi apparatus; and sanguinarine chloride, a Na^+/K^+-ATPase inhibitor [11, 12]. All these five compounds had previously shown less inhibitory or cytotoxic effect in the normal human retinal pigment epithelial cell line hTERT-RPE1 than in the tumor cell lines [11]**.** The four standard drugs were doxorubicin and etoposide which are DNA topoisomerase II inhibitors, oxaliplatin which is alkylating and inhibits DNA synthesis by disrupting DNA replication and transcription, and docetaxel, a microtubule stabilizer and mitotic progression inhibitor. The studies were performed on three neuroendocrine tumor cell lines: the atypical bronchial carcinoid NCI-H720, the typical bronchial carcinoid NCI-H727, and the human pancreatic carcinoid cell line BON-1 wt. Simultaneous drug exposure was evaluated using fluorometric microculture cytotoxicity assay *(*FMCA) [13, 14].

Materials and methods

Cell lines

The human pancreatic carcinoid cell line, BON-1 wt (derived from a lymph node metastasis of a human pancreatic carcinoid tumor) was cultured in a (1:1) nutrient mixture of Dulbecco's Modification of Eagle's Medium (DMEM) and Kaighn's modification medium (F12K) (Invitrogene, Sweden). The human typical bronchial carcinoid cell line NCI-H727 and the human atypical bronchial carcinoid cell line NCI-H720 were obtained from ATCC (LGC Promochem, Sweden) and maintained in RPMI 1640 medium (Invitrogene, Sweden). All three cell lines were supplemented with 10% heat-inactivated fetal calf serum (FCS), 1% glutamine and 1% penicillin/streptomycin (Sigma Aldrich), and cultured in a 5% CO_2-humidified atmosphere at 37°C.

Drugs and plate preparation

The compounds NSC-95397 (dissolved in DMSO), emetine (dissolved in H_2O), CGP-74514A (dissolved in DMSO), brefeldin A (dissolved in ethanol), sanguinarine (dissolved in methanol) as well as the standard drugs etoposide (dissolved in DMSO), oxaliplatin (dissolved in H_2O) and docetaxel (dissolved in PBS) were purchased from Sigma-Aldrich, while doxorubicin (dissolved in PBS) was supplied by the local pharmacy (Uppsala, Sweden). All compounds and drugs were prepared according to the manufacturer's instructions.

The 384-well microtiter plates (Nunclon surface, NUNC Brand Products, Denmark) were pre-prepared with 5 µl drug solution in duplicate at 10 times the desired final drug concentration. Serial drug dilutions and preparations of 384-well microtiter plates were performed by the pipetting robot BIOMek 2000 (Beckman Coulter, USA). The plates were stored at –70°C until use and protected from light during all experimental steps.

Fluorometric microculture cytotoxicity assay (FMCA)

The fluorometric microculture cytotoxicity assay, described in detail previously, was performed to measure cell survival [13, 14]. This method is based on measurement of fluorescence generated from hydrolysis of fluorescein diacetate (FDA) to fluorescein by cells with intact plasma membranes. FDA (Sigma-Aldrich) was dissolved in dimethyl-sulphoxide (DMSO) to 0.5 mg/ml and kept frozen as a stock solution protected from light. Cells were seeded in the drug-prepared 384-well plates using the pipetting robot Precision 2000 (Bio-Tek Instruments Inc., Winooski, VT). The number of cells per well was 5,000. Two columns without drugs served as controls and one column with medium only served as blank. The plates were incubated at 37°C in 5% carbon dioxide for a total incubation time of 72 h. After 72 h incubation at 37°C, medium and drugs were aspirated, the cells washed twice with PBS, 50 µl of physiological buffer, and 1 µl of 0.5 mg/ml FDA were added and after 50–70 min incubation, the fluorescence, which is proportional to the number of living cells, was measured at 485/520 nm in the FLUOstar Optima. Cell survival was presented as survival index (SI) defined

as fluorescence in test wells as a percentage of that in control wells, with blank values subtracted. Quality criteria for a successful assay included a mean coefficient of variation of less than 30% in the control and a fluorescence signal in control wells of more than five times the signal in the blank wells. Only assays which met these criteria are included in the results.

Combination studies

The IC_{50} values (inhibitory concentration 50%), estimated from the log concentration–effect curves in Graph Pad Prism (GraphPad software Inc., CA, USA) using non-linear regression analysis, for all tested drugs in the three cell lines were determined either from the current study or from a previous study [11]. The mean of the estimated IC_{50} of the drug in the three cell lines for each drug was used to select its concentration range for the combination studies.

The combination studies were designed as suggested in the CalcuSyn software manual, using a fixed ratio of the drugs across the concentration range to obtain a good dosage range and dose density [15]. The most efficient way for experimental design is to choose the combination drugs at their equipotent ratio at their IC_{50}′s. After the ratio is set, it is usual to make a mixture of the two drugs of their IC_{50}′s and serially dilute the mixture. The same toxic effect is used for the drugs using the IC_{50}′s. The advantages of the constant ratio design are that each mixture can be treated as a drug to obtain the D_m and m parameters, and for the automated construction of Combination Index (CI) table, CI plot and for the isobolograms. We investigated nine different concentrations of each drugs by diluting the highest concentration twofold to span a wide effect range, on both side of IC_{50}. With this design, the drugs were combined at equipotent concentrations with a fixed ratio. IC_{50} of one drug was combined with IC_{50} of the other drug and so on. Fixed concentration ratios of the drugs were used with twofold serial dilutions in nine steps for combinations and for single drug containing wells. The concentration range for the individual drugs, the mean IC_{50} values for all three cell lines, as well as their drug ratios (test drug/standard drug) are shown in Table 1.

Drug combinations and statistical analysis

To characterize the combination effects between the five compounds and the four standard cytotoxic agents, data were analyzed according to the median-effect method of Chou and Talalay, using the software CalcuSyn Version 2 (Biosoft, Cambridge, UK) [10]. Each dose–response curve (individual agents as well as combinations) was fit to a linear model using the median effect equation, allowing calculation of a median effect value D (corresponding to the IC_{50}) and slope (m). Goodness-of-fit was assessed using the linear correlation coefficient, r, and $r > 0.90$ was required for a successful analysis. The extent of drug interaction between the drugs was expressed using the CI for mutually exclusive drugs:

$$CI = d_1/D_1 + d_2/D_2$$

where D_1 and D_2 represent the concentration of drugs 1 and 2 alone, required to produce a certain effect and d_1 and d_2 are the concentration of drugs 1 and 2 in combination required to produce the same effect. Different CI values are obtained when solving the equation for different effect levels and 75% effect was chosen for presentation. Synergy was defined as CI significantly lower than 1 and antagonism as CI significantly higher than 1. When the confidence interval included 1 the interaction was defined as additive. Statistical analysis was performed using the GraphPad Prism software. Significance level was set to ($p < 0.05$). Comparison of activity between two groups was made with two-sided unpaired Student's t test. One-sample t tests were used to determine if the CI differed from 1.

Results

Table 2 summarizes the combination indexes at an inhibitory effect of 75% for NSC-95397, emetine, CGP-74514, brefeldin A, and sanguinarine combined with the four standard drugs in the three neuroendocrine tumor cell lines.

For the total successful combination analysis (i.e., $r > 0.90$) 38% showed synergism, 52% showed additive effect, and only 10% were scored as antagonistic tested with one-sample t test, $p < 0.05$. The number of synergistic interactions of the five drugs with the four standard drugs in the typical NCI-H727, the atypical NCI-H720, and the pancreatic neuroendocrine cell line BON-1 was 11/20, 7/20, and 5/20 respectively (Table 2).

The combination of NSC-95397 and the four standard cytotoxic drugs showed synergy in the two lung carcinoid cell lines NCI-H727 and NCI-H720 except that docetaxel had additive effect in the atypical carcinoid cell line NCI-H720. The interaction with NSC-95397 and doxorubicin and etoposide in the pancreatic carcinoid cell line BON-1 also showed synergy while it was additive with oxaliplatin and docetaxel. The number of synergistic interactions of NSC-95397 with all four standard drugs was 9/12.

The interactions for emetine were mainly synergy with etoposide, oxaliplatin, and docetaxel while they were additive with doxorubicin in all cell lines. The number of synergistic interactions of emetine with all four standard drugs was 8/12. No antagonistic interactions were reported

Table 1 The tested drugs, concentration range, mean IC_{50}-values for all three neuroendocrine tumor cell lines: pancreatic carcinoid tumor (BON-1), typical broncial carcinoid (NCI-H727) and atypical broncial carcinoid (NCI-H720) and drug ratios

Drug	Concentration range tested (μM)	IC_{50}-value (μM)	Drug ratios (tested drug/standard drug)
NSC-95397	**0.34–86**	**5.4**	
Doxorubicin	0.19–48	3.0	1:0.5
Etoposide	6.6–1,696	106	1:19
Oxaliplatin	2.5–630	39	1:7
Docetaxel	0.62–160	10	1:2
Emetine	**0.0062–1.6**	**0.1**	
Doxorubicin	0.19–48	3.0	1:30
Etoposide	6.6–1,696	106	1:1,060
Oxaliplatin	2.5–630	39	1:394
Docetaxel	0.62–160	10	1:100
CGP-74514A	**0.12–32**	**2.0**	
Doxorubicin	0.19–48	3.0	1:2
Etoposide	6.6–1,696	106	1:53
Oxaliplatin	2.5–630	39	1:20
Docetaxel	0.62–160	10	1:5
BrefeldinA	**0.0062–1.6**	**0.1**	
Doxorubicin	0.19–48	3.0	1:30
Etoposide	6.6–1,696	106	1:1,060
Oxaliplatin	2.5–630	39	1:394
Docetaxel	0.62–160	10	1:100
Sanguinarine	**0.062–16**	**1.0**	
Doxorubicin	0.19–48	3.0	1:3
Etoposide	6.6–1,696	106	1:106
Oxaliplatin	2.5–630	39	1:39
Docetaxel	0.62–160	10	1:10

for NSC-95397 and emetine with the four standard drugs. In Fig. 1, emetine and etoposide combinations showing synergism are visualized as concentration-effect curves, bar graph of chosen concentration, isobolograms and CI plots, in BON-1 (Fig. 1a–d, left panel) and H727 (Fig. 1e–h, right panel) cell lines.

Almost all interactions for CGP-74514A hydrochloride were additive. The synergistic interactions of CGP-74514A, brefeldin A, and sanguinarine chloride with the four standard drugs were few, 1/12, 4/12, and 1/12 respectively, and the number of additive interactions were 10/12, 6/12, and 8/12 respectively. The interactions with etoposide were mainly additive, while the interactions with the other three standard drugs showed a mixed pattern. When interacting with oxaliplatin, sanguinarine showed antagonism in two cell lines and were additive in the remaining cell line. Sanguinarine and oxaliplatin combinations showing antagonism are visualized as concentration–effect curves, bar graph of chosen concentration, isobolograms and CI plots in Fig. 2, in BON-1 (Fig. 2a–d, left panel) and H720 (Fig. 2e–h, right panel) cell lines.

Discussion

The traditional cytotoxic agents are of limited efficacy in the treatment of neuroendocrine tumors and the treatment of such tumors is a significant challenge in oncology. In this in vitro study, we evaluated the combination effect of the five compounds NSC-95397, emetine, CGP-74514A, brefeldin A, and sanguinarine chloride, which have previously shown greater inhibitory effect in neuroendocrine tumor cell lines than in a normal human retinal pigment epithelial cell line [11], with the standard cytotoxic drugs doxorubicin, etoposide, oxaliplatin, and docetaxel in an effort to find drug combinations with potential clinical activity in patients with neuroendocrine tumors. However, performing further experiments using negative control cell lines as human retinal pigment epithelial cell line in the future would lead to gain more understanding about the drug combination toxicity profile. We found that most interaction effects between the five investigated drugs and the four standard cytotoxic agents were synergistic or additive. The Cdc25 dual specificity phosphatase inhibitor NSC-95397 and the protein synthesis inhibitor, and DNA

Table 2 Combination effect of NSC-95397, emetine, CGP-74514A, brefeldin A, and sanguinarine with the standard chemotherapeutic drugs in neuroendocrine tumor cell lines: pancreatic carcinoid tumor (BON-1), typical broncial carcinoid (NCI-H727) and atypical broncial carcinoid (NCI-H720) in vitro

Combination	BON-1 CI at CI_{75}	Effect	NCI-H727 CI at CI_{75}	Effect	NCI-H720 CI at CI_{75}	Effect
NSC-95397 + Doxorubicin	0.65 (0.39–0.91)	Synergistic	0.49 (0.32–0.67)	Synergistic	0.57 (0.20–0.93)	Synergistic
NSC-95397 + Etoposide	0.54 (0.39–0.69)	Synergistic	0.80 (0.73–0.86)	Synergistic	0.51 (0.23–0.80)	Synergistic
NSC-95397 + Oxaliplatin	0.92 (0.73–1.1)	Additive	0.62 (0.46–0.78)	Synergistic	0.66 (0.46–0.85)	Synergistic
NSC-95397 + Docetaxel	0.83 (0.64–1.0)	Additive	0.65 (0.56–0.73)	Synergistic	0.79 (0.48–1.1)	Additive
Emetine + Doxorubicin	0.92 (0.55–1.3)	Additive	1.5 (0.23–2.8)	Additive	0.97 (0.83–1.1)	Additive
Emetine + Etoposide	0.46 (0.32–0.59)	Synergistic	0.63 (0.54–0.72)	Synergistic	0.74 (0.68–0.81)	Synergistic
Emetine + Oxaliplatin	0.50 (0.47–0.54)	Synergistic	0.77 (0.36–1.2)	Additive	0.58 (0.35–0.81)	Synergistic
Emetine + Docetaxel	0.48 (0.36–0.60)	Synergistic	0.70 (0.48–0.91)	Synergistic	0.66 (0.39–0.92)	Synergistic
CGP-74514A + Doxorubicin	1.1 (0.99–1.2)	Additive	0.61 (0.55–0.68)	Synergistic	0.98 (0.70–1.3)	Additive
CGP-74514A + Etoposide	0.87 (0.70–1.0)	Additive	0.84 (0.62–1.1)	Additive	1.3 (0.95–1.7)	Additive
CGP-74514A + Oxaliplatin	0.88 (0.72–1.0)	Additive	0.92 (0.47–1.4)	Additive	0.79 (0.48–1.1)	Additive
CGP-74514A + Docetaxel	1.0 (0.94–1.1)	Additive	1.1 (1.0–1.3)	Antagonistic	1.3 (0.38–2.2)	Additive
Brefeldin A + Doxorubicin	0.83 (0.64–1.0)	Additive	0.61 (0.42–0.80)	Synergistic	1.7 (1.4–2.1)	Antagonistic
Brefeldin A + Etoposide	1.0 (0.89–1.1)	Additive	0.68 (0.64–0.71)	Synergistic	0.69 (0.43–0.96)	Synergistic
Brefeldin A + Oxaliplatin	0.97 (0.90–1.0)	Additive	1.0 (0.89–1.2)	Additive	1.4 (0.37–2.5)	Additive
Brefeldin A + Docetaxel	1.1 (1.0–1.1)	Antagonistic	0.62 (0.28 –0.96)	Synergistic	1.0 (0.82–1.2)	Additive
Sanguinarine + Doxorubicin	1.1 (0.97–1.3)	Additive	0.52 (0.46–0.59)	Synergistic	1.1 (0.84–1.3)	Additive
Sanguinarine + Etoposide	1.1 (0.73–1.4)	Additive	1.2 (0.99–1.4)	Additive	1.5 (0.95–2.1)	Additive
Sanguinarine + Oxaliplatin	1.4 (1.0–1.7)	Antagonistic	1.2 (0.99–1.5)	Additive	1.6 (1.1–2.2)	Antagonistic
Sanguinarine + Docetaxel	1.1 (0.64–1.6)	Additive	1.1 (0.79–1.4)	Additive	1.7 (1.6–1.8)	Antagonistic

Synergism and antagonism are defined as mean of CI at CI_{75} with 95% confidence interval statistically significantly lower/higher than 1, tested with one-sample t test ($p > 0.05$). Additive effect is defined as 95% confidence interval including 1

interacting agent emetine had the most attractive interactions (synergy) in combination with the four standard cytotoxic drugs in the three cell lines tested. Both NSC-95397 and emetine scored 6 out of the 11 synergy interactions in the typical bronchial carcinoid and 6 out of the 7 synergy interactions in the atypical bronchial carcinoid cell line. In addition, they scored all of the 5 synergy interactions of the five compounds with the four standard drugs in the pancreatic carcinoid cell line. The synergy interactions of NSC-95397 and emetine in the pancreatic and atypical bronchial carcinoid cell lines are worth noting as the pancreatic carcinoid cell line previously has been shown to be the least sensitive cell line to the five drugs [11] and clinical studies have demonstrated a worse prognosis for patients with atypical compared to patients with typical bronchial carcinoids [16].

Clinical data have shown that streptozotocin-based combinations including doxorubicin and 5-flourouracil generated partial remissions in patients with pancreatic endocrine tumors, and that etoposide combined with cisplatin may be active in patients with high proliferative pancreatic endocrine tumors or lung carcinoids [17, 18]. All NSC-95397 combinations with doxorubicin and etoposide were synergy which may indicate that these combinations may be the possible candidates for further pre-clinical and clinical studies. Docetaxel is a drug not commonly used in patients with carcinoids, and there have been no published reports, preclinical or clinical, on docetaxel-containing combinations in the treatment of patients with neuroendocrine tumors. However, in a case reported by Warner a patient with typical midgut carcinoid responded very well to docetaxel treatment [19], and treatment of metastatic carcinoid tumor patients with the taxanes docetaxel and paclitaxel has resulted in biochemical responses but no significant antitumor activity [20, 21]. It has previously been reported that NSC-95397 showed synergy effect with paclitaxel in the presence of dexamethasone in breast cancer cell line [22] and that the combination of other Cdc25 inhibitors with paclitaxel have additive effect on colon cancer cells [23]. Our results indicate that docetaxel as well as oxaliplatin are suitable candidates for combination with NSC-95397, showing synergistic and additive effects. It is important to remember that additive interactions, not only synergistic, could be of clinical benefit if the drugs have non-overlapping toxicity profiles. Docetaxel's known toxicity is bone marrow suppression, and the toxicity induced by oxaliplatin includes neuropathy and neutropenia. NSC-95397 is a

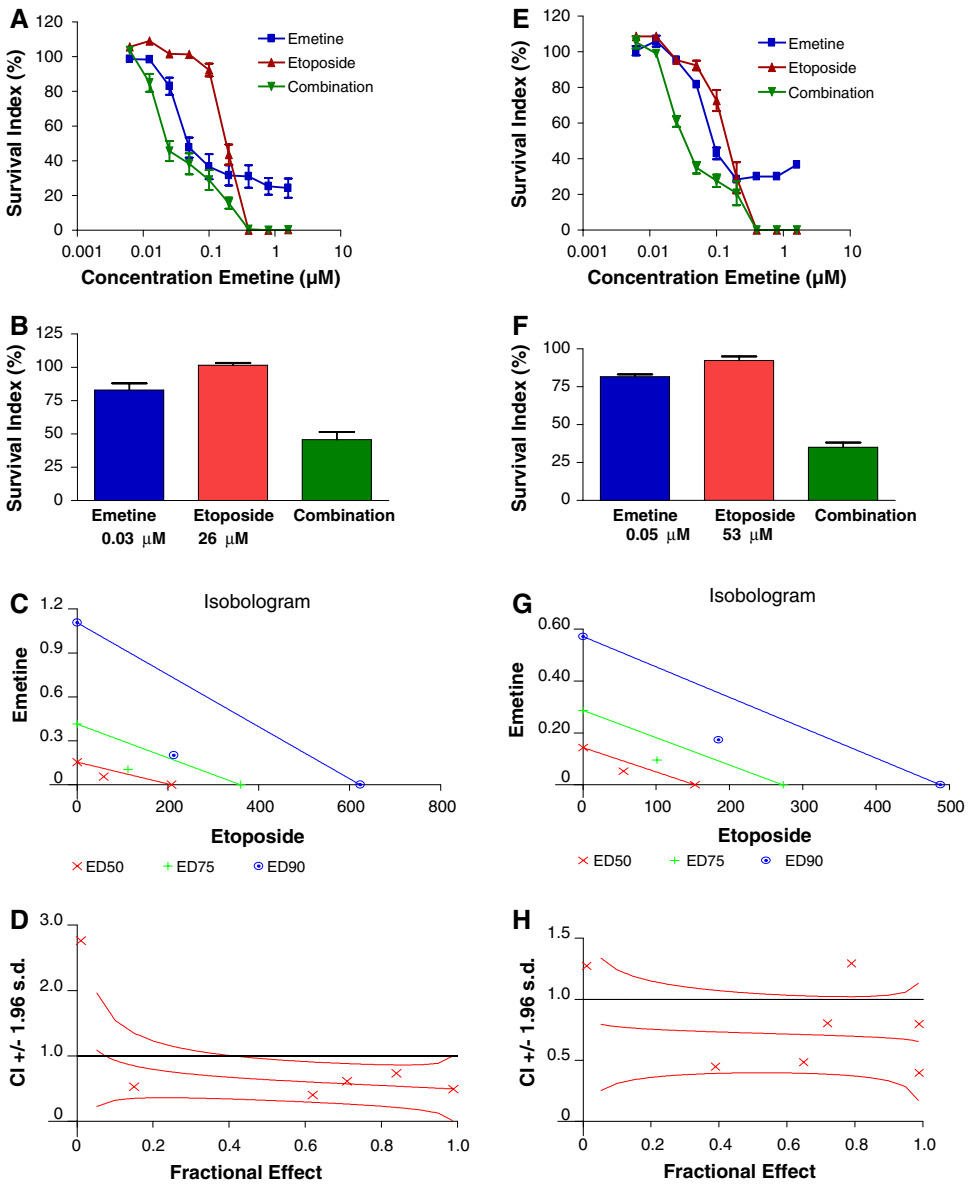

Fig. 1 Combination of emetine and etoposide in the human pancreatic carcinoid cell line, BON-1(**a**–**d**, *left panel*) and in the human typical bronchial carcinoid cell line NCI-H727 (**e**–**h**, *right panel*). First row (**a**, **e**) shows the concentration effect curves, IC_{50}'s: emetine 0.1 μM, etoposide 106 μM, x-axis shows the concentration for emetine and the ratio for emetine–etoposide is 1:1,060. The second row, (**b**, **f**) shows a bar graph of chosen concentration. The results are from the FMCA analysis. Third row (**c**, **g**) shows the isobolograms at 50, 75, and 90% effect level and fourth row (**d**, **h**) the simulated combination index values (CI) with the 95% confidence interval at all effect levels as calculated by the CalcuSyn software

naphthoquinone compound and no reports are available regarding its toxic side effects. Many naphthoquinone derivatives, however, are hemolytic agents. It is possible that these compounds might not have overlapping toxicity profiles with docetaxel and oxaliplatin. Combining drugs with synergistic or additive interaction effects and non-overlapping side effects may enable dose reduction of toxic chemotherapeutic agents, yet maintaining enough antitumor effect.

In our study, all emetine combinations with etoposide, docetaxel, and oxaliplatin were synergy except with oxaliplatin in the typical carcinoid cell line which was additive. Such combinations are thus interesting objects for further studies. Earlier publications reported that the combination of emetine with the alkylating agent cyclophosphamide for treatment of lung cancer patients has shown a definite response [24], and with cisplatin in leukaemia cells has additive effect [25]. The interaction of emetine with doxorubicin was additive. Since emetine is cardio toxic [26], it is however not suitable to combine with doxorubicin which is well known for its cardiac side effects. The human multidrug transporter P-glycoprotein limits the accumulation of drugs in HIV-infected cells and cancer cells. The inhibition of this drug transporter can revise the drug resistance and increase the bioavailability of chemotherapeutic drugs. Recently, emetine has been reported to work as a P-glycoprotein inhibitor [27]. The synergistic and the additive effect we found in the combinations with emetine can thus at least in part be explained by inhibition of P-glycoprotein.

Almost all of the interactions with the cyclin-dependent kinase-1 inhibitor CGP-74514A and the standard cytotoxic

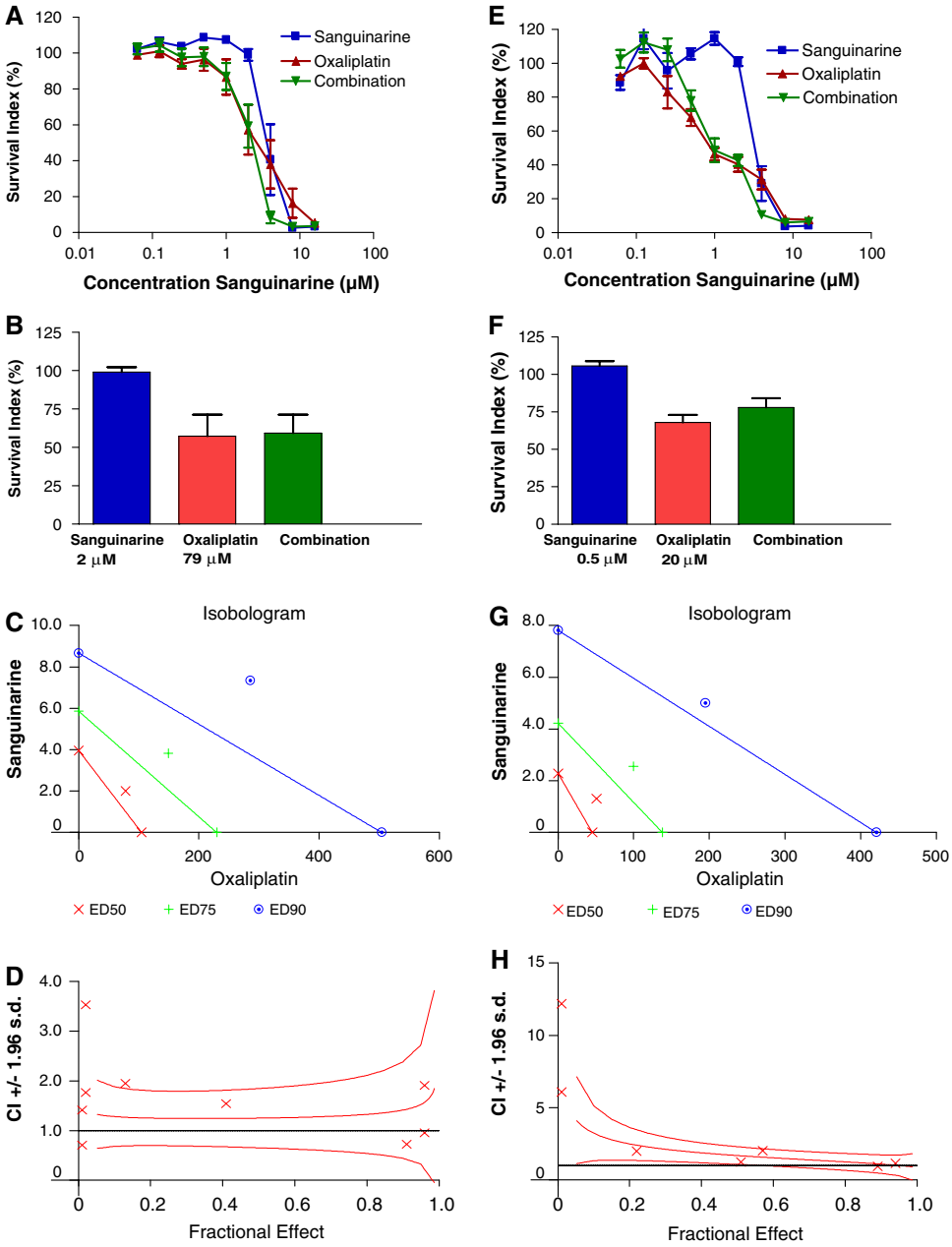

Fig. 2 Combination of sanguinarine and oxaliplatin in the human pancreatic carcinoid cell line, BON-1 (**a**–**d**, *left panel*) and in the human atypical bronchial carcinoid cell line NCI-H720 (**e**–**h**, *right panel*). First row (**a**, **e**) shows the concentration effect curves, IC_{50}′s: sanguinarine 1.0 μM, oxaliplatin 39 μM, x-axis shows the concentration for sanguinarine and the ratio for sanguinarine–oxaliplatin is 1:39. The second row (**b**, **f**) shows a bar graph of chosen concentration, both resulting from the FMCA analysis. Third row (**c**, **g**) displays the isobolograms at 50, 75, and 90% effect level and fourth row (**d**, **h**) shows the simulated combination index values (CI) with the 95% confidence interval at all effect levels as calculated by the CalcuSyn software

drugs were additive. This agent may thus also be a possible candidate for further studies. The two remaining compounds, brefeldin A and sanguinarine displayed less synergy interactions, with combination indices above 1 in most of the interactions with the standard drugs. Thus, they seem less attractive to combine with the standard chemotherapeutic agents we tested. Notably, sanguinarine was antagonistic in two and additive in the third cell line when interacting with oxaliplatin. This may be explained by their similar mechanism of action. Oxaliplatin inhibits DNA synthesis by disrupting DNA replication and transcription. Sanguinarine has also been found to bind to DNA as well as with core histones, thus inducing chromatin aggregation [28].

In the current study, we explored the potentiation of simultaneous exposure which seems to be promising. It would also be interesting to study sequential exposure with different time schemes and frames to explore the influence of sequence dependency on the potentiation of the combination effect between NSC-95397 and emetine, and standard agents.

In conclusion, the evidence of synergistic and additive effects reveals potential advantages of incorporating NSC-95397, emetine and CGP-74514A into combination chemotherapy regimens and these drugs might be the suitable candidates for clinical studies in patients with bronchial carcinoids and pancreatic endocrine tumors.

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Conflict of interest statement No potential conflicts of interest were disclosed.

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