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난소암 및 자궁경부암 세포주에 있어서 Topotecan의 시험관내 상호 작용

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=Abstract=

Effect of Topotecan in Combination with Other Antitumor Drugs in Vitro

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Objectives: The aims of this study were to evaluate the interaction of topotecan with adriamycin, etoposide, 5 fluorouracil (5 FU) and mitomycin C in the established four ovarian cancer cell lines and three cervical cancer cell lines and to establish whether the combination of topotecan with other antitumor drugs would be a synergism for chemotherapy in patients with ovarian and cervical cancer.

Methods: Five antitumor drugs were tested for synergism and antagonism in combination studies in four ovarian cancer cell lines (A2780, A2780 cisplatin resistant variant, A2780 taxol resistant variant, SKOV3) and three cervical cancer cell lines (HeLa, SiHa, ME180). Cytotoxic effects were determined by MTT assay. Synergic interaction was calculated by the median effect principle in which combination index (CI) of less than one suggest a synergic interaction.

Results: Dm value of topotecan against SKOV3 (2.07 ug/ml), HeLa (3.32 ug/ml), SiHa cell lines (2.5 ug/ml) were above peak plasma concentration of topotecan (0.5 ug/ml) but most antitumor drugs tested in combinations index were within clinically relevant range. Combination with topotecan showed a synergic effect (CI<1) in seven cancer cell lines at a intermediate or high level of cytotoxicity especially with mitomycin C (6/7), etoposide (6/7), 5 FU (6/7) and adriamycin (4/7). Most striking findings were that a synergic effect was shown in all ovarian cancer cell lines to topotecan/mitomycin C, topotecan/5 FU and topotecan/esoposide combination showed a synergic effect in all cervical cancer cell lines. Topotecan/adriamycin combination showed synergism at an intermediate or high level of cytotoxicity in cisplatin or Taxol resistant ovarian cancer cell lines (A2780CP, A2780TX, SKOV3).

Conclusion: These results suggested that topotecan showed a synergic effect with a wide range of antitumor drugs: adriamycin, etoposide, 5 FU, mitomycin C in ovarian and cervical cancer cell lines. Combinations of topotecan/mitomycin C, topotecan/5 FU and topotecan/adriamycin for ovarian cancer and a combination of topotecan/etoposide for cervical cancer seemed worthy of consideration for clinical application.

Key Words: Topotecan, Combination index, Synergism, Antagonism

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Introduction

Most patients with ovarian cancer present with advanced disease. Optimal debulking operation followed by platinum-based chemotherapy has improved response rates and, to a lesser degree prolonged survival.[1] However most patients will relapse and be candidates for further palliative chemotherapy. Systemic therapy for carcinoma of the cervix is recurrent primary cancer or distant metastasis.[2] Few agents have demonstrated any major activity in cisplatin resistance to ovarian cancer and recurrent cervical cancer.[3,4]

A recent comparative study has shown that first line chemotherapy with cisplatin and taxol in advanced ovarian cancer is superior to cisplatin and cyclophosphamide and a combination of cisplatin/paclitaxel or carboplatin/paclitaxel is becoming the first line of chemotherapy.[5-7]

As taxol moves to the first line of chemotherapy there is a critical need to identify a new agent for second line chemotherapy in advanced ovarian cancer. Patients with recurrent cervical cancer have a poor prognosis with one-year survival rate of less than 15% and few antitumor drugs are active in the cervical cancer.[2,8] The aim of this study was to assess synergism and antagonism of combinations of topotecan with adriamycin, etoposide, 5 Fluorouracil and mitomycin C and to establish a potential candidate for secondary line of topotecan base combination chemotherapy in ovarian and cervical cancer. Synergism and antagonism in chemotherapy were measured with the median effect principle and the combination index isobologram method.[9-11]

Material and Methods

Cell culture

The human ovarian and cervical cancer cell lines were grown to confluence in tissue culture flasks using RPMI 1640 media for A2780, A2780CP (cisplatin resistant variant), A2780TX (Taxol resistant variant) and DMEM for HeLa, SiHa and McCoy's 5 media for SKOV3, ME180 with 10% fetal calf serum (Gibco. USA) in an atmosphere 5% CO_2 at 37℃ Penicillin (100 U/ml) and Streptomycin (100 ug/ml) and Fungizon were added to the media.

Cytotoxicity assays

Adriamycin, etoposide, 5 Fluorouracil (5FU), and mitomycin C were obtained from the Sigma Chemical Co.(St. Louis, MO, USA). Topotecan was provided by Smithkline Beecham Pharmaceuticals, Philadelphia, PA. Adriamycin, 5FU, mitomycin C and Topotecan were made as 1 mg/ml stock with media. Etoposide was dissolve with DMSO. All cytotoxocity experiments were performed more than twice in triplicate samples.

Cell preparation from culture in the confluence phase were remove from culture flask by trypsin (0.05%) with EDTA(0.02%) for 10 minutes at 37℃. The cell were then centrifuged. Cells were put into 96 well at a concentration of 1-2 × 105/ml. The cells were allowed to adhere to bottoms of plate for 24 hours, then the drug of fresh medium was added to well in a serial dilution of 1/2. The concentration of drugs corresponded to 0.25, 0.5, 1, 2, 4 times for a fixed dose of drugs (Dm of A drug: Dm of B drug). The plates were incubated under the same condition for 72 hours. After drug incubation for 72 hours, cell proliferation was determined by 3-(4,5-dimethylthiazol-2-ly)-2,5-diphenyltetrazolium bromide (MTT) assay (CellTiter96TM). Twenty ul of MTT solutions were added to each well. After 4 hours at 37℃, 100 ul of stop solution were added and then overnight incubation for solubilizing the MTT-formazan product. The plates were read at an absorbance at 570 nm on a 96 well microplate reader (Anthos Ht III).

Median effect principle for Dose effect analysis

The multiple drug effect analysis of Chou and Talalay which is based on the median effect principle was used to calculate combined drugs effect.[9-11]

$fa/(1-fa) = (D/Dm)^m$

In this equation, D is the dose, Dm is the dose required for 50% effect of cytotoxicity, fa is the fraction affected by dose D, fu is the unaffected fraction (fu=1-fa) and m is a coefficient of the sigmoidicity of the dose-effect curve: m=1, m>1, m<1 indicate hyperbolic, sigmoidal and negative sigmoidal curve effect, respectively.

$D = Dm(fa/(1-fa))^{1/m}$

The Dm and m value are determined by the median-effect plot: x=log(D), y=log(fa/fu), m is the slope and log(Dm) is x intercept. For level of cytotoxicity(f=0.95,0.9,0.85,0.8,0.75,,,,,0.05) the combination index(CI) was calculated.

$CI=(D)1/(Df)1+(D)2/(Df)2+\alpha(D)1(D)2/(Df)1(Df)2$

CI<1, CI=1, CI>1 indicated synergism, addictive effect and antagonism, respectively. $\alpha=1$, $\alpha=0$ depending on whether the drugs are assumed to be mutually nonexclusive or mutually exclusive, respectively. For simplicity a mutual exclusive assumption has been widely used but we assumed between topotecan and other antitumor drugs mutually nonexclusive.

Results

Single drug parameters

Dm is the concentration of drug required to produce the median effect (50% survival fraction). In all cases the data results in a linear plot with regression coefficients over 0.9. This level of correlation is required to appropriately use this model to assay synergy. Our study showed variable range of the Dm value in the ovarian and cervical cancer cell lines(Table 1). Dm value of topotecan against SKOV3 (2.07 ug/ml), HeLa (3.32 ug/ml), SiHa cell lines (2.5 ug/ml) were above peak plasma concentration of topotecan (0.5 ug/ml) and Dm value of adriamycin against SKOV3 was above the peak plasma concentration of adriamycin (0.6 ug/ml). Most antitumor drugs tested in combinations index were within a clinically relevant range at: adriamycin(6/7), etoposide(7/7), 5FU (7/7), mitomycin C(7/7), topotecan(4/7).(Table 1)

Interaction between topotecan and adriamycin

The CI calculated on the assumption of mutually nonexclusive is drawn as a solid line in Fig. 1 and Fig. 5. Dose concentrations of topotecan/adriamycin combination were within the range of clinically relevant in A2780, A2780CP, SKOV3, A2780TX, HeLa, ME180

Table 1. Dm[a] value of Various Chemotherapeutic Agents In four Human ovarian And three Cervical cancer cell lines

Adriamycin		Etoposide	5 FU	MitomycinC	Topotecan
Ovarian cancer cell lines					
A2780	0.004	1.01	0.85	0.008	0.004
A2780CP	0.046	3.42	2.26	0.064	0.19
A2780TX	0.07	2.52	2.4	0.026	0.026
SKOV3	0.84[b]	8.97	25.69	0.658	2.07[b]
Cervical cancer cell lines					
HeLa	0.42	26.58	20.06	0.82	3.32[b]
SiHa	0.36	10.04	7.36	0.95	2.5[b]
ME180	0.13	3.29	17.76	0.31	0.069

[a] Dm=ug/ml. Concentration of drug required to cause a 50% inhibition of cell growth.
[b] Dm value are over peak plasma concentration (0.6 ug/ml Adriamycin, 34.2 ug/ml Etoposide, 60 ug/ml 5 FU, 1.5 ug/ml Mitomycin C, 0.5 ug/ml Topotecan)17).

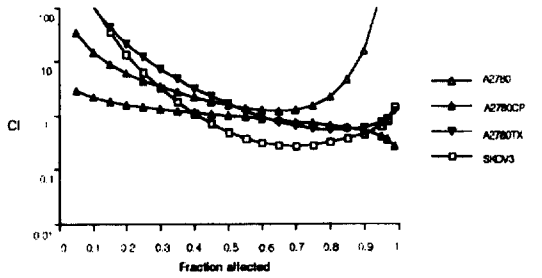

Fig 1. Combination effect of Topotecan and Adriamycin

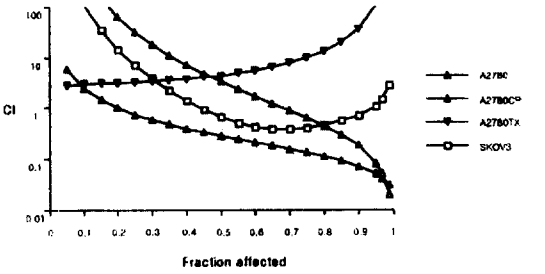

Fig 2. Combination effect of Topotecan and Etoposide

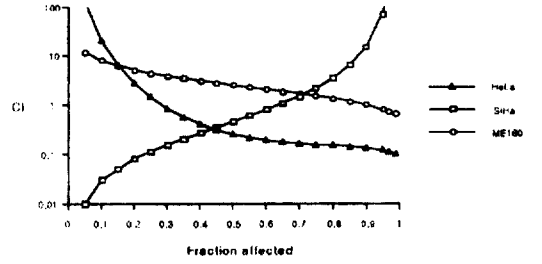

Fig 5. Combination effect of Topotecan and Adriamycin

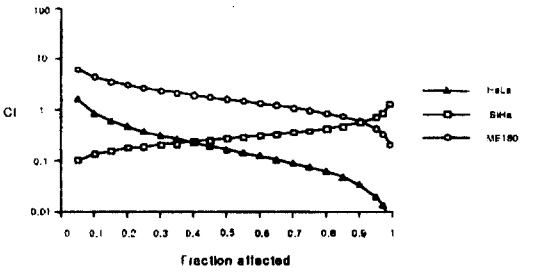

Fig 6. Combination effect of Topotecan and Etoposide

cell lines(6/7). Topotecan/adriamycin combination showed synergism in 3/4th ovarian cancer cell lines and topotecan/adriamycin resistant SKOV3 cell lines showed synergism but topotecan/adriamycin sensitive A2780 cell lines were antagonistic in all ranges of cytotoxocity. Topotecan/adriamycin combination showed synergism in 2/3s of cervical cancer cell lines at a high level of cytotoxicity. Topotecan/adriamycin combination showed synergism at high level of cytotoxicity in five out of seven cell lines tested.

Interaction between topotecan and etoposide

Dose concentrations of topotecan/etoposide combination were within the range of clinically relevant in A2780, A2780CP, SKOV3, A2780TX, HeLa, ME180 cell lines (6/7). Topotecan resistant SKOV3, HeLa and SiHa cell lines (3/3) showed a synergic effect at intermediate or high level of cytotoxicity. Topotecan/etoposide combination showed synergism in 3/4 ovarian cancer cell lines and all cervical cancer cell lines tested. Topotecan/etoposide combination showed synergism at intermediate or high level of cytotoxicity in six out of seven cell lines tested.(Fig. 2, 6)

Interaction between topotecan and 5 FU

Dose concentrations of topotecan/5 FU combination were within the range of clinically relevant in A2780, A2780CP, SKOV3, A2780TX, HeLa, ME180 cell lines (6/7). Topotecan resistant SKOV3, HeLa and SiHa cell lines (3/3) showed synergic effect at intermediate or high level of cytotoxicity for topotecan/5 FU combination. Topotecan/5 FU combination showed synergism in all ovarian cancer cell lines tested and 2/3s of cervical cancer cell lines tested. Topotecan/5 FU combination showed synergism at intermediate or high level of cytotoxicity in six out of seven cell lines tested.(Fig. 3, 7)

Interaction between topotecan and mitomycin C

Dose concentrations of topotecan/mitomycin C combination were within the range of clinically relevant in A2780, A2780CP, SKOV3, A2780TX, HeLa, ME180

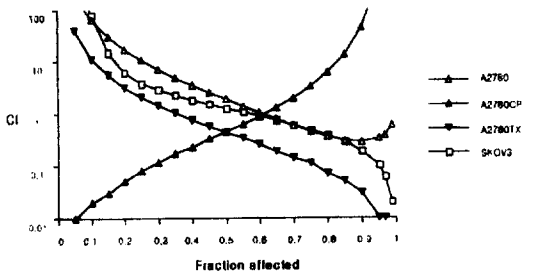

Fig 3. Combination effect of Topotecan and 5 FU

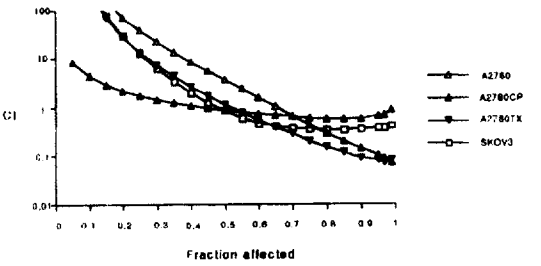

Fig 4. Combination effect of Topotecan and Mitomycin C

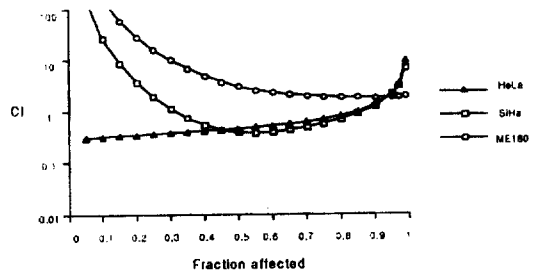

Fig 7. Combination effect of Topotecan and 5 FU

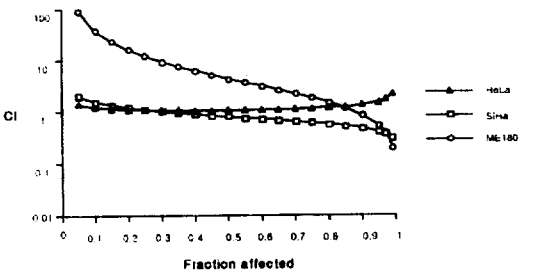

Fig 8. Combination effect of Topotecan and Mitomycin C

cell lines (6/7). Topotecan resistant SKOV3, HeLa and SiHa cell lines (2/3) showed a synergic effect at intermediate and high level of cytotoxicity for topotecan/mitomycin C combination. Topotecan/mitomycin C combination showed synergism in all ovarian cancer cell lines tested and 2/3s of cervical cancer cell lines tested. Topotecan/mitomycin C combination showed synergism at intermediate or high level of cytotoxicity in six out of seven cell lines tested.(Fig. 4, 8)

Discussion

Over the last decade, platinum-based combination chemotherapy regimens have led to higher response rates and longer survival for advanced ovarian cancer patients than previous regimens based on alkylating agents. The advent of paclitaxel for salvage therapy, patients receiving the paclitaxel combination had a higher overall response rate, a longer time to disease progression, and prolonged median survival.[1] Nonetheless, even with current treatments, relapse rates remain high and most women with advanced ovarian cancer ultimately will die of their disease. For this reason, the development of new, effective second-line treatments, as well as better first-line agents, for advanced disease remains a high priority. To maximize the efficacy of second- or third- line drugs, new agents should be non-cross-resistant with platinum or paclitaxel. Chemotherapy drugs for advanced ovarian cancer with novel mechanisms of action include topotecan (Hycamtin; SmithKline Beecham Pharmaceuticals, Philadelphis, PA), a topopisomerase I inhibiter. Topotecan was recently shown to be effective in platinum-refractory of -resistant patients, with response rates ranging from 14% to 23%.[13] Although radiation therapy and surgery form the basis for treatment of cervical cancer limited to the pelvis, those who have advanced disease or recurrences after locoregional therapy depend on systemic treatment for any hope of disease control. No chemotherapy for advanced or recurrent carcinoma of the cervix is more effective than single-agent cisplatin. The major thrust of current and future investigation seeks to identify additional

active agents and to develop combinations that offer greater patient benefit.[2] In recurrent squamous carcinoma of the cervix, irinotecan has an objective response rate of 15%-24%.[14] Our study was to evaluate the effect of topotecan combination in the established four ovarian cancer cell lines and three cervical cancer cell lines and to establish whether the combination of topotecan with other antitumor drugs would be synergism for chemotherapy in patients with ovarian and cervical cancer and to obtain a potential candidate for a therapeutic regimen. We used the established human cancer cell lines to evaluate the effect of new agents or combination of agents because they give the direction to select chemotherapuetic drugs. There were several methods of evaluating the interaction of drug combinations but there was no standard method to predict exact interaction.[15]

We calculated the interaction of drugs by median effect principle but there were some limitations that data for each cell line were made using a fixed ratio of each pair of drugs and some cell lines showed the Dm level was above peak plasma concentration at standard doses. The results of CI can be changed to a different ratio of drugs and may not be clinically relevant.[16] There were poor correlations between the plasma concentration and it's intracellular concentration in vitro but one study demonstrated a significant relationship between toxicity in vitro and achievable systemic exposure of anticancer drugs in humans.[17]

Moreover this technique may not reflect the exact clinical situation and does not give therapeutic results such as toxicities to the tumor compared with toxicity to the host. However, we studied the combination effect of chemotherapy by cytotoxicity assay in vitro and can predict the common results which were not a specific effect of one cell line in directing combination chemotherapy against seven ovarian and cervical cancer cell lines. Even though drug concentration used in vitro test can't reflect or parallel to serum plasma concentration, dose concentrations used in combination index were clinically relevant except SiHa cell lines(6/7). Our studies showed three topotecan resistant cell lines which showed synergic effect with etoposide (3/3), 5 FU (3/3), adriamycin (2/3) and mitomycin C (2/3) but mechanisms between the topotecan resistant cell line and synergic were unknown. In various studies assessed CPT-11 has been found to have synergism and no cross resistance with cisplatin, cytosine arabinose or mitomycin C and an addictive effect with adriamycin, etoposide and 5 FU.[15,18] Another study showed topotecan combination effect can be varied depending on the cell line type being tested.[16] Our studies showed topotecan had synergism against a wide range of drugs tested not for a particular drug: seven cancer cell lines at an intermediate or high level of cytotoxicity especially with mitomycin C (6/7), etoposide (6/7), 5 FU (6/7) and adriamycin (4/7). The cellular mechanism behind these synergisms remains to be determined. Interesting findings were topotecan/adriamycin combination showed synergism at an intermediate or high level of cytotoxicity in cisplatin or taxol resistant ovarian cancer cell lines (A2780PC, A2780TX, SKOV3). Our data's suggested combinations with topotecan/mitomycin C, topotecan/5 FU and topotecan/adriamycin were suited for secondary lines of chemotherapy against ovarian cancer and clinical trials using these agents are warranted to determine if there are any survival advantages over other drugs.

Multiple studies have documented improved partial response rates with platinum-based multiagent chemotherapy for recurrent cervical cancer and the most effective nonplatinum agents appear to be doxorubicin, ifosofmide, mitolactol, and vincristine.[8] But no regimen has been associated with an improved survival duration. To improve the poor prognosis of this patient group, identification of new agents with at least equivalent activity to cisplatin is mandatory. The cervical cancer cell lines we tested showed them relatively resistant to chemotherapy compare with ovarian cancer cell lines. Topotecan/etoposide combination (topoisomerase I inhibitor/topoisomerase II inhibitor) showed synergic in all cervical cancer cell lines tested. While the mechanism of these synergisms is still controversy, these agents inhibit Topoisomerase

II by trapping a covalent enzyme - DNA cleavage complex.[19,20] Moreover, topotecan/adriamycin, topotecan/5 FU, and topotecan/mitomycin C combinations showed synergism in 2/3s of cervical cancer cell lines at a high level of cytotoxicity. Our studies showed a combination of topotecan/etoposide for cervical cancer seemed worthy of consideration for clinical application.

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국문초록

목적: 확립된 4종류의 난소암 세포주와 3종류의 자궁경부암 세포주에 있어서 topotecan 과 각각의 adriamycin, etoposide, 5 FU, mitomycin과의 상호 작용시 상승작용 혹은 길항작용을 알아보고 향후 난소암 혹은 자궁경부암환자에 상승 작용이 있는 조합을 이용함으로써 임상적인 유용성을 기대할 수 있다.

재료 및 방법: 5 종류의 항 암화학제와 4 종류의 난소암세포주(A1780, cisplatin 내성이 있는 A2780CP, Taxol에 내성이 있는 A2780TX, SKOV3)와 3 종류의 자궁경부암 세포주(ME180, SiHa, HeLa)를 이용하였다. 세포주성장 억제 측정은 MTT assay 로 하였고 상승작용은 "median effect principle" 을 이용하여 조합지수(combination index)가 1 이하 인경우 상승 작용으로 판단 하였다.

결과: topotecan의 Dm 치(50% 세포억제 농도)는 SKOV3(2.07ug/ml), HeLa(3.32ug/ml), SiHa (2.5ug/ml)에서 혈장 최고치보다 높았으나 대다수의 세포주에서는 임상적인 효과 범위내의 혈장치내에 속하였다. Topotecan과 조합시 7개의 암세포주 중 mitomycin (6/7), etoposide(6/7), 5FU(6/7), adriamycin(4/7)에서 상승 작용효과를 보였으며 topotecan/mitomycin, topotecan/5 FU 조합이 모든 난소암 세포주에 있어서 상승 작용을 보였으며 topotecan/etoposide 조합이 모든 자궁경부암 세포주에 상승작용을 보였다. 또한 cisplatin 이나 taxol에 저항 있는 세포주(A2780CP, 2780TX, SKOV3)에서 topotecan/adriamycin 조합이 특히 상승작용을 관찰하였다.

결론: topotecan은 난소암이나 자궁경부암에 있어서 adriamycin, etoposide, 5 FU, mitomycin 등과 상승작용을 관찰할 수 있었으며 향후 임상적으로 난소암환자에서는 topotecan/mitomycin과 topotecan/5FU, topotecan/adriamycin 조합을 자궁경부암 환자에서는 topotecan/etoposide를 조합시 상승 작용을 기대 할 수 있으리라 생각된다.