Synergistic Growth-Inhibitory Effects of Fenretinide with Either Cisplatin or Paclitaxel on Human Epithelial Ovarian Cancer Cell Line (SKOV-3)

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Objectives : *To study the growth-inhibitory effects of cisplatin, paclitaxel and fenretinide on human epithelial ovarian cancer cell line (SKOV-3) and to determine whether fenretinide can synergy with the first two drugs.*
Study design: *Experimental study*
Material and Method : *Human epithelial ovarian cancer cell line (SKOV-3) was cultured. Different concentrations of cisplatin, paclitaxel and fenretinide were added into cells. The LD_{50} concentraion of each drug was measured. The number of viable cells was determined by MTT (dimethyl thiozolyl-2 ,5 -diphenyl-2-H-tetrazolium bromide) assay. The interactions between fenretinide-cisplatin and fenretinide-paclitaxel were represented as percent inhibition of viable cells.*
Results : *The LD_{50} concentrations of cisplatin, paclitaxel and fenretinide were 1.5 g/ml, 27 nmol/ml and 0.4 mol/ml, respectively. The percent inhibition of viable cells of cisplatin was 35%, 70% and 74% (at 1, 2 and 2.5 g/ml), paclitaxel was 5%, 9% and 43% (at 5, 10 and 20 nmol/ml) and fenretinide was 9%, 12% and 25% (at 0.025, 0.05 and 0.1 mol/ml), respectively. The growth-inhibitory effects of the cisplatin-fenretinide combination: 1+0.025, 2+0.05 and 2.5+0.1 and paclitaxel-fenretinide combination: 5+0.025, 10+0.05 and 20+0.1 were 100% with statistically significance . These combinations of fenretinide with either cisplatin or paclitaxel demonstrated the synergistic growth-inhibitory effects.*
Conclusion : *Combinations of fenretinide with either cisplatin or paclitaxel demonstrated the synergistic growth-inhibitory effects. From our results, we expected that the using of fenretinide in combination with cisplatin or paclitaxel can possibly lower the dosage of these drugs. Therefore, the side effects and toxicities of drugs could be reduced.*

Keywords : *Cisplatin, Paclitaxel, Fenretinide, SKOV-3, Synergistic growth-inhibitory effects*

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Ovarian cancer carries the worst prognosis among gynecologic cancers because it is rarely diagnosed at early stage and aggressively progresses when diagnosed. Although surgery and adjuvant chemotherapy is the treatment of choice, overall 5 year survival rates is only 20-30%[1] . The commonly used chemotherapeutic drugs, cisplatin and/or paclitaxel possibly cause serious side effects due to their toxicities. Therefore, the development of new drugs is required for the optimal treatment with less drug resistance and furthermore less toxicities[1].

Fenretinide is a vitamin-A derivatives that exerts potent influences on cell differentiation, proliferation, homeostasis and development. In previous reports, fenretinide has been suggested for clinical usage in ovarian cancer treatment according to its growth-inhibitory effect and apoptosis induction[2]. Additionally, in previous studies, fenretinide seemed to be well tolerated with only minimal or mild toxicity depending on the dose used [2-4].

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In this study, we sought to investigate the growth-inhibitory effects of cisplatin, paclitaxel and fenretinide on human epithelial ovarian cancer cell line (SKOV-3) and to determine whether fenretinide could synergy with these drugs.

Material and Method

Cell line and culture condition

Human epithelial ovarian cancer cell line used in our study was SKOV-3. It was obtained from American Type Culture Collection (ATCC). The cell line was routinely maintained in RPMI 1640 (Gibco Co.) supplemented with 5% fetal bovine serum (FBS) (Gibco Co.).

Cells were grown in 75 cm^2 tissue culture flask. Cell preparations from culture in log-phase growth were removed from the culture flask by trypsinization. Cells were seeded into 96-well tissue culture plates in 100 l of RPMI-5% FBS culture medium at the concentration of $4x10^3$ cells per well. After 24 hours from seeding, 100 l of cisplatin, paclitaxel or fenretinide were added into the culture medium and the cells were further grown for additional 24 hours in 5% CO_2 incubator at 37 C. Cisplatin concentrations were 0, 1, 1.5, 2, 2.5 and 3 g/ml, paclitaxel concentrations were 0, 5, 10, 20, 27 and 50 nmol/ml and fenretinide concentrations were 0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.175, 0.2, 0.3, 0.4, 0.5, 0.8 and 1 mol/ml. Each drug concentration was added into four wells. Cell growth was measured 2 times, the first at 24- hour intervals and the second at 48-hours intervals by MTT (dimethyl thiozolyl-2 ,5 -diphenyl-2-H-tetrazolium bromide) based cell proliferation assay followed by 20% SDS. Interpretation of MTT based cell proliferation assay was based upon the basis by which MTT (yellow color) may convert to the blue color called "purple formazan product" that produced by reduction of MTT by succinyl dehydrogenase in the mitochondria of viable cells. The plate containing MTT uptake cells was placed in the microplate reader. Optical densities (OD) of viable cells were measured at a wavelength of 570 nm (OD data not shown). Number of viable cells was then calculated. The LD_{50} concentration (concentration that necessary to yield a 50% inhibition of measured growth) of cisplatin, paclitaxel and fenretinide were measured.

Each data point of drug concentration represented the mean value calculated from four wells in one experiment. All experiments were done three times.

Synergistic effect analysis

For the synergistic effects analysis, concentrations of each drug were selected at the doses below the LD_{50} concentration. Then combinations of fenretinide with either cisplatin or paclitaxel (cisplatin-fenretinide combination : 1+0.025, 2+0.05 and 2.5+0.1 paclitaxel-fenretinide combination : 5+0.025, 10+0.05 and 20+0.1) were added into cells in tissue culture plates , 8 wells per each combination step by step as described above and cells viability were assessed by MTT based cell proliferation assay followed by 20% SDS, 48 hours thereafter.

The interactions between fenretinide-cisplatin and fenretinide-paclitaxel were determined by fractional inhibition method as follows: additive inhibition produced by both inhibitors ($i_{1,2}$) occurs when $i_{1,2} = i_1 + i_2$; synergism when $i_{1,2} > i_1 + i_2$; and antagonism when $i_{1,2} < i_1 + i_2$. Fractional inhibition of cell viability was represented as percent inhibition of viable cells which can be calculated by this formula[5].

Percent inhibition of viable cells =[1-(viable cells after drug treatment /untreated cells)]x100

Statistical analysis

Statistical comparison of mean values of percent inhibition of viable cells between cisplatin or fenretinide alone and cisplatin-fenretinide combination, paclitaxel or fenretinide alone and paclitaxel-fenretinide combination were performed using Student's t test. Statistically significant was determined when p-value was less than 0.05.

Results

Fractional inhibition of cell viability by cisplatin, paclitaxel and fenretinide on human ovarian cancer cell line (SKOV-3) were represented as percent inhibition of viable cells and the results of various sensitivities to cisplatin , paclitaxel and fenretinide were shown in Fig. 1, 2 and 3, respectively. LD_{50}'s as determined by the MTT assay were as follows: cisplatin 1.5 g/ml, paclitaxel 27 nmol/ml and fenretinide 0.4 mol/ml. The percent inhibition of viable cells in the presence of cisplatin alone at 1, 2 and 2.5 g/ml was 35%, 70% and 74%, in the presence of paclitaxel alone at 5, 10 and 20 nmol/ml was 5%, 9% and 43% and in the presence of fenretinide alone at 0.025, 0.05 and 0.1 mol/ml was 9%, 12% and 25%. In the determination of synergistic growth-inhibitory effects, the combinations of fenretinide-cisplatin and fenretinide-paclitaxel were added into the SKOV-3 cell. Different doses of fenretinide (0.1, 0.05 and 0.025 mol/ml), cisplatin (1, 2 and 2.5 g/ml) and paclitaxel (5, 10 and

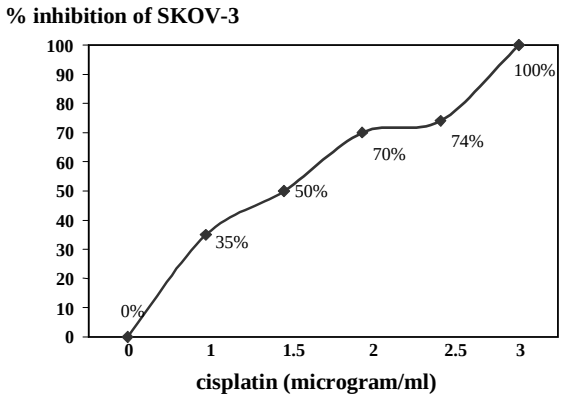

Fig. 1 Percent inhibition of SKOV-3 cell in the presence of cisplatin alone

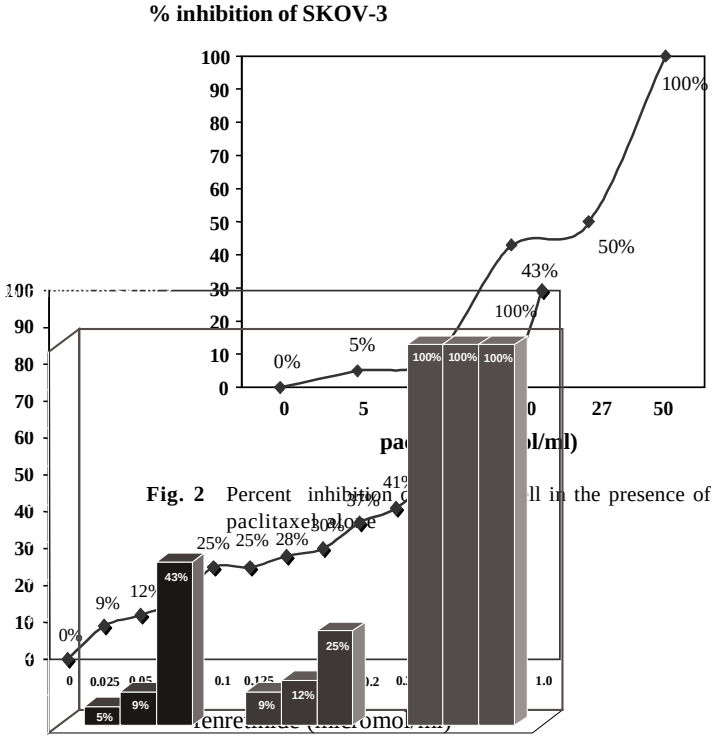

Fig. 2 Percent inhibition of SKOV-3 cell in the presence of paclitaxel alone

Fig. 3 Percent inhibition of SKOV-3 cell in the presence of fenretinide alone

20 nmol/ml) were combined and the results of percent inhibition of viable cells were demonstrated in Fig. 4 and 5. All cisplatin-fenretinide and paclitaxel-fenretinide combinations induced a 100% synergistic growth-inhibition. The statistically significant increase of percent inhibition of viable cells compared between individual drugs and their combinations were also demonstrated ($p < 0.001$).

Discussion

Fenretinide, a synthetic vitamin-A derivatives is an antioxidant. It may decrease risk of cancer occurrence by limiting oxidative DNA damage by reactive

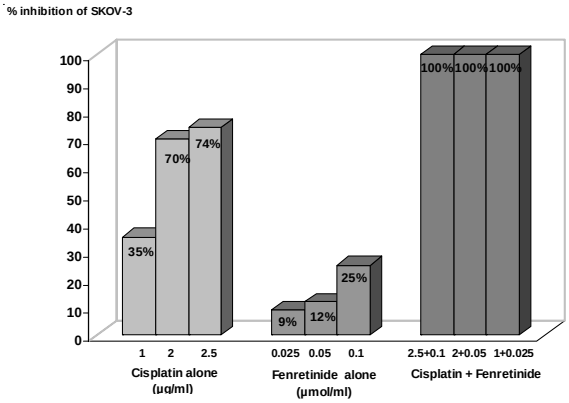

Fig. 4 Synergistic growth-inhibitory effects comparing between cisplatin or fenretinide alone and cisplatin-fenretinide combination

Fig. 5 Synergistic growth-inhibitory effects comparing between paclitaxel or fenretinide alone and paclitaxel-fenretinide combination

oxygen species (ROS) that leading to cancer initiation[2] . It seems that fenretinide has significantly different properties when compared with other natural retinoids such as all-trans-retinoic acid (ATRA) and 9-cis retinoic acid in terms of its mode of storage and plasma half-life. The main difference is the absence of hepatic accumulation of fenretinide, implying reduced liver toxicity. Other side effects of natural retinoids such as nyctalopia, photophobia, cheilitis and pruritus were not observed when using fenretinide. Moreover, the terminal plasma half-life of fenretinide is found to be 12 hours which is much longer than natural retinoid, ATRA. This finding may also make fenretinide more preferable[2]. Recently, a number of studies have suggested that fenretinide may play a potential role as an ovarian cancer chemotherapeutic agent[8,9]. It inhibits the growth of human ovarian cancer cells both in vitro and in vivo with fewer side effects reported[8,9].

In previous phase I/II trials, fenretinide seemed to be well tolerated with only minimal or mild toxicity depending on the dose used[6]. There are currently many clinical trials from the National Cancer Institute of USA reported the recommended dose of fenretinide orally, 200 mg per day[6].

In our study, we aimed to investigate whether fenretinide can synergy with commonly used chemotherapeutic drugs (cisplatin and paclitaxel) on human epithelial ovarian cancer cell line (SKOV-3). Our results demonstrated the growth inhibitory effects of cisplatin, paclitaxel and fenretinide on SKOV-3 cell line which were similar to the previous report[8,11]. However, in our study, the LD_{50} of these drugs on SKOV-3 cell line were lower than those of the previously reported[11]. Our LD_{50} of cisplatin and paclitaxel were 1.5 g/ml and 27 nmol/ml, respectively compared to those from Gibb et al[11]: cisplatin 5 g/ml, and paclitaxel 100 nmol/ml. For fenretinide, our LD_{50} was only 0.4 mol/ml, which was also lower than the previously reported clinically achievable concentration, 1 mol/ml[6,7].

The reasons for these differences were not certainly determined but we hypothesized that they were possibly due to the cell growing conditions such as the culture medium, passages of SKOV-3 cell line and variation on drugs .

Data from another study about dosage of fenretinide for growth inhibition of human ovarian cancer cell line demonstrated that when ovarian cancer cell lines (including SKOV-3) were treated with a concentration of 1 μmol/ml (a pharmacologically concentration which previous pharmacokinetic study demonstrating that these concentration is obtainable with 200 mg oral administration), fenretinide inhibits most effectively the growth of ovarian cancer cell lines[9]. From our study, it may be implicated that using fenretinide at lower dosage (0.4 μmol/ml) could effectively inhibit SKOV-3 cell growth as well as the higher dosage (1 μmol/ml), so the toxicities and side effects of drug may be lowered. Equivalent oral form of this lower dosage (0.4 μmol/ml) could be further studied.

Additionally, we found that when fenretinide at the concentration below the LD_{50} (0.025, 0.05 and 0.1 mol/ml) were combined with either cisplatin (1, 2 and 2.5 g/ml) or paclitaxel (5, 10 and 20 nmol/ml), the synergistic effects of 100% growth inhibition were both demonstrated and these findings also supported our hypothesis about its synergistic growth-inhibitory effects. Therefore, we expected that the using of fenretinide in combination with cisplatin or paclitaxel could help in reduction of the dosage of these chemotherapy, so the toxicities and side effects could be minimized. Further studies on the appropriate concentrations of fenretinide combined with other chemotherapeutic agents aiming to reduce doses and toxicities for clinical applications need to be developed.

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ผลการเสริมฤทธิ์ของสารเฟนเรติไนด์กับยาซิสพลาตินและแพคลิแทกเซลในการยับยั้งการเจริญเติบโตของเซลล์ไลน์มะเร็งรังไข่ของมนุษย์ชนิดเยื่อบุผิว SKOV-3

ชุตินาท อิ่มเอม, สายบัว ชี้เจริญ, ประสิทธิ์ เรืองไรรัตนโรจน์, ทิพวรรณ เลียบสื่อตระกูล

วัตถุประสงค์ *: เพื่อศึกษาผลของยาซิสพลาติน, แพคลิแทกเซล และสารเฟนเรติไนด์ในการยับยั้งการเจริญเติบโตของเซลล์ไลน์มะเร็งรังไข่ของมนุษย์ชนิดเยื่อบุผิว (SKOV-3) และดูผลการเสริมฤทธิ์ของสารเฟนเรติไนด์กับยาซิสพลาตินและแพคลิแทกเซลในการยับยั้งการเจริญเติบโตของเซลล์ดังกล่าว*

รูปแบบการศึกษา *: การวิจัยเชิงทดลองในห้องปฏิบัติการ*

วิธีการศึกษา *: เพาะเลี้ยงเซลล์ไลน์มะเร็งรังไข่ของมนุษย์ชนิดเยื่อบุผิว (SKOV-3) ในน้ำยาเพาะเลี้ยงเซลล์จนได้ปริมาณที่เหมาะสม จากนั้นนำสารเฟนเรติไนด์, ยาซิสพลาตินและแพคลิแทกเซลในความเข้มข้นต่าง ๆ ใส่ลงไปในน้ำยาเพาะเลี้ยงเซลล์ วัดค่า LD_{50} และปริมาณเซลล์ที่ยังมีชีวิตอยู่หลังจากได้รับยาดังกล่าวโดยใช้ MTT assay คำนวณหาค่าเปอร์เซ็นต์การยับยั้งการเจริญเติบโตของเซลล์เพื่อดูความสัมพันธ์ของปฏิกริยาระหว่างสารเฟนเรติไนด์-ซิสพลาติน และเฟนเรติไนด์แพคลิแทกเซล ในเซลล์ไลน์ดังกล่าว*

ผลการศึกษา *: ค่า LD_{50} ของยาซิสพลาติน, แพคลิแทกเซล และสารเฟนเรติไนด์ เท่ากับ 1.5 g/ml, 27 nmol/ml และ 0.4 mol/ml ตามลำดับ เปอร์เซนต์การยับยั้งการเจริญเติบโตของเซลล์ของซิสพลาติน เท่ากับ 35%, 70% และ 74% เมื่อใช้ยาที่ความเข้มข้น 1, 2 และ 2.5 g/ml, แพคลิแทกเซล เท่ากับ 5%, 9% และ 43% เมื่อใช้ยาที่ความเข้มข้น 5, 10 และ 20 nmol/ml และเฟนเรติไนด์ เท่ากับ 9%, 12% และ 25% เมื่อใช้ยาที่ความเข้มข้น 0.025, 0.05 และ 0.1 mol/ml ตามลำดับ ศึกษาผลการยับยั้งการเจริญเติบโตของเซลล์เมื่อนำยา 2 ชนิดมารวมกัน โดยเมื่อนำยาซิสพลาตินมารวมกับสารเฟนเรติไนด์ตามความเข้มข้น: 1+0.025, 2+0.05 และ 2.5+0.1, และเมื่อนำยาแพคลิแทกเซลมารวมกับสารเฟนเรติไนด์ตามความเข้มข้น: 5+0.025,10+0.05 และ 20+0.1 พบว่าการนำยาดังกล่าวมาใช้ร่วมกันสามารถยับยั้งการเจริญเติบโตของเซลล์ไลน์มะเร็งรังไข่ของมนุษย์ชนิดเยื่อบุผิว (SKOV-3) ได้อย่างมีนัยสำคัญทางสถิติ ร้อยละ 100 และการนำสารเฟนเรติไนด์มารวมกับยาซิสพลาตินและแพคลิแทกเซลสามารถเสริมฤทธิ์ในการยับยั้งการเจริญเติบโตของเซลล์ไลน์มะเร็งรังไข่ของมนุษย์ชนิดเยื่อบุผิว (SKOV-3) ได้*

สรุป *: การนำสารเฟนเรติไนด์มารวมกับยาซิสพลาตินและแพคลิแทกเซลสามารถเสริมฤทธิ์การยับยั้งการเจริญเติบโตของเซลล์ไลน์มะเร็งรังไข่ของมนุษย์ชนิดเยื่อบุผิว (SKOV-3) ได้จากผลการทดลองดังกล่าวคณะผู้วิจัยคาดว่า การนำสารเฟนเรติไนด์มาใช้ร่วมกับยาซิสพลาตินและแพคลิแทกเซล อาจจะช่วยลดขนาดของยาเคมีบำบัด (ซิสพลาตินและแพคลิแทกเซล) ลง ดังนั้นผลข้างเคียงและพิษของยาเคมีบำบัดน่าจะลดลง*