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The Effect of Verapamil on Chemosensitivity by 5-Fluorouracil and Cisplatin in Human Uterine Cervical Carcinoma Cell Lines

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Abstract: Verapamil , a potent calcium channel blocker, has been proved to be one of the modulators to overcome drug resistance in cancer chemotherapy. In the present experiment, the possibility of verapamil as a MDR modulator was investigated by using MTT assay. Sole treatment of verapamil on the HeLa and CasKi cervical cancer cell line revealed dose dependent cytotoxicity within a range of tested dose. Combined treatment of verapamil with 5-FU, DDP on two human cervical cancer cell line led to a significant synergistic cytotoxicity. Therefore , these studies showed that verapamil had a possibility to be applicable to cancer chemotherapy in gynecological oncology.

Key words: Verapamil, MTT, Cervical cancer cell line, 5-FU, DDP

INTRODUCTION

Cervical cancer is a first cause of death due to cancers in Korean women and seems to be rapidly increasing[9]. Furthermore, invasive cervical cancer is regarded as a systemic disease rather than localized lesion[2,8]. Thus, surgery or radiation therapy alone little contributes to overcome advanced cervical cancer[10]. Also, many patients with cervical cancer do not well respond to chemotherapy though chemotherapy is still one of the effective treatments[4]. The problems in chemotherapy of cervical cancer are that one is a limitation in use as higher dosage of cytotoxic drugs and another is a acquisition of resistance induced by the cytotoxic agents on tumor cells[11,12]. Recently, some clinical studies using P glycoprotein inhibitors to modulate drug resistance have been done and a few promising results have been reported[1,7,13,15]. However, the clinical use of verapamil in the field of cervical cancer has been poorly documented. Therefore, it is important to judge on whether verapamil can be used as a possible adjuvant of chemotherapy. Thus, the present study was undertaken to evaluate if verapamil potentiate cytotoxcity of 5-Fluorouracil(5-FU) or Cisplatin(DDP) in chemosensitivity test using human uterine cervix cancer cell lines *in vitro*.

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MATERIALS AND METHODS

Drugs

Verapamil(VRM) and other chemicals were

obtained from Sigma Co., Ltd(St. Louis, USA).

Cancer cell lines

The cancer cell lines for cytotoxicity test were as follows: HeLa (uterine cervical cancer, human), CasKi (uterine cervical cancer human). Each cell line was maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and incubated in a humidified 5% CO_2 chamber at 37℃.

Measurement of cytotoxicity

To evaluate cytotoxicity, modified MTT method was performed essentially as described previously[3,6]. Briefly, monocellular susupension was seeded at 10^4 cells per well in 96 well plates with 100 μl of medium per well. To examine synergism between verapamil and cytotoxic agents(5-FU, DDP), they were added at varying concentrations and cultures were incubated for 72 hours in an incubator maintaining a highly humidified atmosphere, 5% CO_2 and 95% air. Fifty μl of the medium containing MTT(5mg/ml) were added to each well. After 4 hours of exposure, the medium was removed and the wells were washed with PBS, and then 50 μl of DMSO were added to each well to solubilize the precipitates. The plates were transferred to an ELISA reader to measure absorbance at 570nm. 50% inhibitory concentration(IC_{50})value, which means 50% inhibition of cell growth, was calculated by regression analysis (plotting the viablity versus the concentration of the test compound). All experiments were done at least 3 times, with 4 wells for each concentrations of test agents.

RESULTS AND DISCUSSION

To explore the clinical applicability of verapamil, we first examined the effect of verapamil on tested cell lines in vitro. Sole treatment of verapamil on the HeLa and CasKi cervical cancer cell line revealed dose dependent cytotoxicity within a range of tested dose(Fig 1, Fig 4). IC_{50} of verapamil on tested cell lines is 103.3 μg/ml in Hela cell and 55.49 μg/ml in CasKi cell, respectively(Table 1). These concentration-effect curve patterns by verapamil on tested cell line were similar. In particular, it was noted that verapamil alone may be cytotoxic to tested cell lines(Fig 1, Fig 4). This result was considered to be relevant with the report that verapamil alone had an antiproliferative effect on brain tumor cells *in vitro*[14]. Thirty μg/ml of verapamil or less had little influence on the value of cytotoxicities. Thus, we adopted 5 to 10 μg/ml of verapamil for MTT test in synergism. According to our results, IC_{50} of 5-FU on HeLa cell line could be decreased to 0.07458 to 0.1356μg/ml than IC_{50} (0.8262μg/ml) without verapamil if 5 to 10μg/ml of verapamil is added (Fig 3, Table 1). This phenomenon was also observed in a combined treatment of verapamil and DDP on HeLa cell line(Fig 2) and also turned out to be statistically significant. In CasKi cell, IC_{50} of verapamil is about half as that of verapamil on

Table 1. 50% inhibitory concentration[a] following sole or combined treatment of verapamil, 5-FU and DDP in HeLa and CasKi cell line

	IC_{50} (μg/ml) of Drug						
Cell lines	VRM	DDP	DDP +VRM(5)[b]	DDP +VRM(10)[c]	5FU	5FU +VRM(5)	5FU +VRM(10)
HeLa	103.3	5.279	0.2482	3.882	0.8262	0.1356	0.07458
CaSki	55.49	3.083	1.850	0.9688	7.121	7.112	10.63

a: Measured by MTT assay.
b, c: VRM(5 μg/ml), VRM(10 μg/ml) which was added to DDP or 5-FU.

HeLa cell line(Table 1). Combined effect of 5-FU and verapamil was stronger than that of DDP plus verapamil on CasKi cell line(Fig 5, Fig 6 and Table 1). Generally, in CasKi cell line, the modulation by verapamil could be interpretated to be minimal and statistically insignificant compared to in HeLa cell(Table 1).

In cervical cancer cell line, there is little report about synergism between verapamil and cytoticity agents. On the test of synergy, the enhancement of cytotoxicity by verpamil was occured in all tested cell lines at varying degree. But, it is not obvious that this result is due to direct modulation of P glycoprotein (P gp) pump or MRP related action by verapamil. To elucidate this synergism by verapamil, the molecular apparoach using the anti P-gp antibody or drug efflux assay is essentially ne-

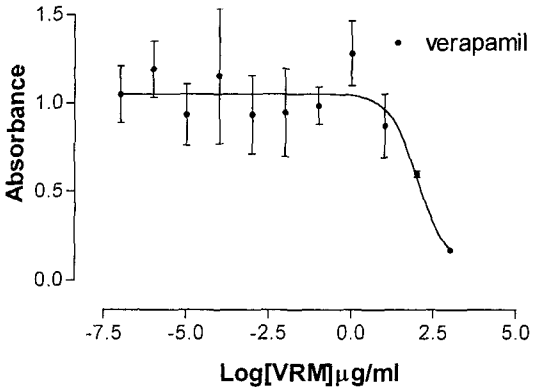

Fig. 1. The survival curve of HeLa cell by verapamil.

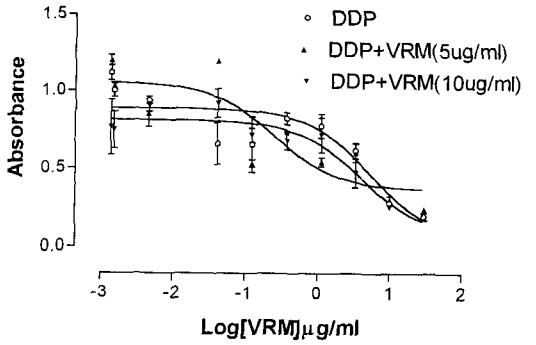

Fig. 2. The survival curve of HeLa cell by DDP+ verapamil.

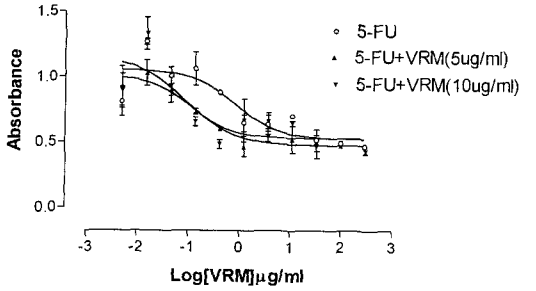

Fig. 3. The survival curve of HeLa cell by 5FU+ verapamil.

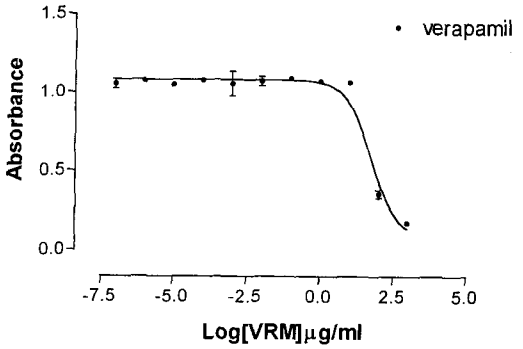

Fig. 4. The survival curve of CaSki cell by verapamil.

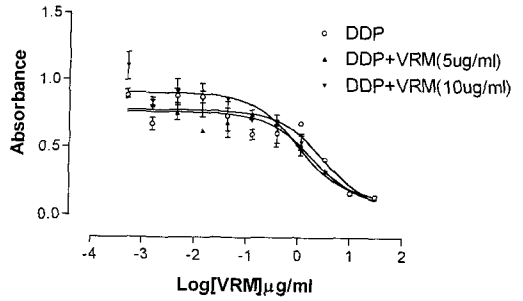

Fig. 5. The survival curve of CaSki cell by DDP+verapamil.

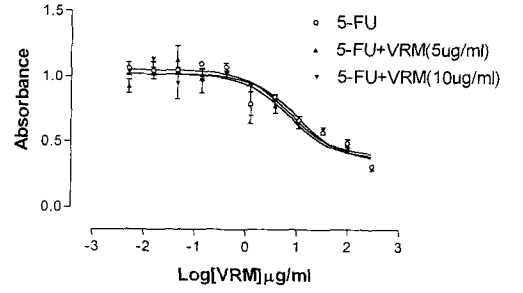

Fig. 6. The survival curve of CaSki cell by 5FU+ verapamil.

eded. Meanwhile, one explanation for different potentiation may be a result of inconsistency in histologic cell type between tested two cell lines. That is why HeLa cell is originated from glandular cell and CasKi cell from epithelial cell. Another hypothesis may be an involvement of other resistance mechanism (topoisomerase, glutathione-related enzymes) in interacting manner. Recently, it was reported that mitoxantrone-resistant HeLa cells displayed not only a lower level of cleavage acitivity but also of topoisomerase II content and catalytic activity, relative to the parental drug-sensitive HeLa cells[5]. Therefore, the potentiaton of cytotoxicity by verapamil can be supposed to associate with a regulatory action on topoisomerase II. Now, further study is going on progress to analyze the potentiation effect by verapamil on these cell lines.

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=국문초록=

Verapamil의 인체 자궁경부암 세포주에서 5-FU 및 Cisplatin 감수성에 관한 효과

연세대 원주의대 산부인과학교실[1], 연세대 원주의대 미생물학교실[2] 및 기초의학연구소[3]

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Verapamil은 항암제에 대한 내성을 극복하기 위하여 실험적으로 사용하는 대표적인 약제로 알려져 있다. 본 연구에서는 부인과 종양중 자궁경부암 치료시에 발생하는 항암화학요법제에 대한 내성을 극복하기 위한 기초적인 실험으로 인체 자궁경부암 유래의 HeLa 및 CasKi 세포주를 이용, MTT법으로 verapamil과 5-FU 및 DDP를 단독 혹은 병용처리하여 세포독성 상승효과를 측정하였다.-- Verapamil 단독투여시 각 세포주는 용량에 비례하여 세포독성이 증가하였으며, 각 항암제와의 병용투여시에 세포독성의 상승효과가 관찰이 되었으며, HeLa세포주에서 그 효과가 가장 높았다. 이러한 결과를 바탕으로 세포내 내성발현의 기전의 탐구가 필요하며 자궁경부암 화학요법에 verapamil사용의 이론적인 가능성을 제시하고 있다.

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