Expression of multidrug resistance (MDR) & effect of MDR inhibitor activity in renal cell carcinoma

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Expression of multidrug resistance (MDR) & effect of MDR inhibitor activity in renal cell carcinoma

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ABSTRACT

Expression of multidrug resistance (MDR) & effect of MDR inhibitor activity in renal cell carcinoma

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Purpose: Renal cell carcinoma (RCC) is instrisically resistant to chemotherapy, because 80-90% of RCC express multidrug resistance (MDR)1 as energy-dependent efflux pump. 1B3PY1 is newly synthesized MDR1 inhibitor designed based on other MDR1 modulator-verapamil. In this study, we investigated the expression of MDR1 in Caki-2 RCC cell line and human RCC cell that was obtained from the patients, then

each combination effect of taxol,doxorubicin with 1B3PY1 in cultured Caki-2 cell and human RCC cell.

Materials and methods: Caki-2 RCC cell line, and clinical samples were obtained from 8 males and 2 females with conventional clear cell RCC who had metastatic lesions at operation. After cell culute of specimens, Expression of MDR1 in Caki-2 cell and human RCC cell was estimated by RT-PCR. Cytotoxicity of 1B3PY1 was investigated, and then combination effect of taxol, doxorubicin with 1B3PY1 in Caki-2 cell and human RCC cell were evaluated.

Results: MDR1 was expressed in Caki-2 cell and human RCC cell. The MDR1 in human RCC cell was significantly higher than that of Caki-2 cell. New MDR1 inhibitor, 1B3PY1 revealed no cytotoxicity in Caki-2 cell and human RCC cell. Compared with IC_{50} of taxol or doxorubicin only, there were also no significant combination effect with taxol or doxorubicin and 1B3PY1 in Caki-2 cell. In human RCC cell, there was also no significant combination effect with taxol and 1B3PY1, whereas IC_{50} in group of doxorubicin and 1B3PY1 was significantly decreased compared with that of doxorubicin only, maximum difference of which was upto 19 times.

Conclusions: As a new MDR1 inhibitor, we suggest IB3PY1 would give a perspective in the treatment of metastatic RCC. Further study will be needed.

Key words: renal cell carcinoma, multidrug resistance, chemotherapy, taxol, doxorubicin

Expression of multidrug resistance (MDR) & effect of MDR inhibitor activity in renal cell carcinoma

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I. INTRODUCTION

Kidney cancer accounts for approximately 2% of all adult cancers. It is the 7th leading cause of cancer in the United States with an estimated incidence of approximately 51,000 new cancer cases per year in 2007.[1] The incidence of kidney cancer is rising; it is 2 times more common in men than women.[2] The 2007 survey by Korean national cancer institute stated that the incidence registration rate of kidney cancer was 2.3% of all cancer occurred in men, which was 10th in order. Renal cell

carcinoma (RCC) is the most common kidney tumor and it is arising from the cells in the lining of tubules in the kidney.[3] The incidence of RCC has been increasing steadily over the past decades. The diagnostic trend is mainly due to the widespread use of non-invasive abdominal imaging procedures, which detect incidental renal lesions.[4] The majority of these incidentally detected tumors are at low stages and low grades and are armenable to curative surgical treatments; therefore carry a good prognosis.[5,6] However, a stable proportion of 20% to 30% of patients still present with metastatic disease, and 20% to 30% of the patients who undergo curative surgery will develop metastatic disease during follow-up.[7] Systemic metastases portend a particularly poor prognosis for RCC, with 1-year survival of less than 50%, 5-year survival of 5% to 30%, and 10-year survival of 0% to 5%.[8-10]

One common theme that has persisted is that RCC remains primarily a surgical disease-it is still considered the paradigm of the chemorefractory tumor. It is well known that RCC patients often show poor or partial response to chemotherapy and the mechanism is only partially explained. Multidrug resistance (MDR) is the principal mechanism by which many cancers develop resistance to chemotherapeutic drugs. It affects patients with a variety of blood cancers and solid tumors, including breast,

ovary, lung and gastrointestinal tract cancers.[11] Human RCC displays a high degree of intrinsic drug resistance, and patient's response to chemotherapeutic treatment is poor.[12,13] Increased expression of the membrane-associated P-170 glycoprotein, a product of the MDR1 gene, has been associated with the multidrug-resistant phenotype,[14-16] and there is evidence that P-170 acts primarily as an ATP dependent pump which exports drug molecules across the cellular membrane.[17] Therefore, resistance which is associated with a P-glycoprotein such as P-170 is characterized by a decrease in intracellular accumulation and retention of drug.[15,18,19] Virtually every other cytotoxic agent has been tested for this disease, either alone or in combination with other chemotherapeutic approaches, almost uniformly with discouraging results. Currently available data of chemotherapy do not demonstrate reproducible antitumor activity or improvement in survival of patients treated for metastatic clear cell carcinoma.[20]

The inherent resistance of RCC to conventional treatment has lead to the use of immunotherapies such as alpha-interferon and interleukin-2. However, response rates of immunotherapy for RCC have been disappointing, typically ranging from 15% to 20% despite a variety of creative treatment strategies, suggesting immune

tolerance.[9,21] Newer "targeted-therapies" such as sunitinib are emerging for the treatment of patients resistant/intolerant to current treatment modalities or as an alternative to cytokine immunotherapy. Unfortunately, median progression- free survival was 11.0 months in metastatic RCC.[22,23]

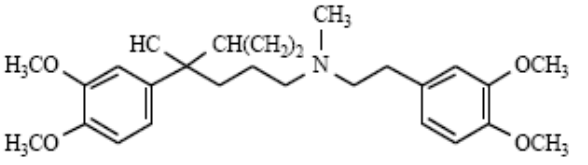
Chemotherapy is the standard treatment for most solid tumors; however, RCC is generally resistant to chemotherapy, largely because of their intrinsic chemo-resistance by MDR. Therefore, if we could overcome this obstacle, we would adopt chemotherapy as a major treatment modality in RCC same as the other solid tumors.

Attempts to modulate MDR were judged particularly relevant to RCC since there is nearly uniform expression of P-glycoprotein on these cells. MDR reversal agents were studied in some clinical trials for RCC in combination with vinblastine or doxorubicin.[24-30] None was shown to enhance an antitumor effect. Moreover, the response rate to vinblastine alone or with a modulating agent in these more recent trials was 3% in 277 patients.[24,26-31] This lack of antitumor activity demonstrates that vinblastine is ineffective and emphasizes the need for new insight into overcoming drug resistance.

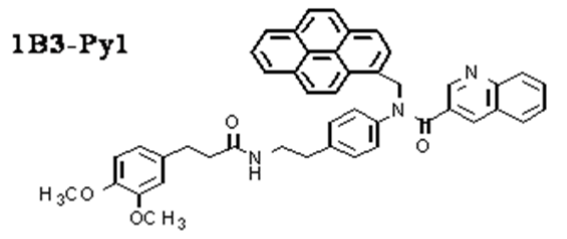
We now synthethized new type of MDR inhibitor and named that as 1B3PY1.

1B3PY1 was initially designed based on previously developed other MDR modulator-verapamil (Fig. 1).

Fig. 1. The comparison of structure: verapamil and 1B3PY1.



Verapamil
(phaenylalkylamines)



1B3PY1 had the typical characteristics of 3rd generation MDR inhibitor. In previous study performed in haman uterine sarcoma cell line, MES-SA (MX2), expressing MDR1, the combination therapy with taxol and 1B3PY1 reduced IC_{50} upto 400 folds compared with that of taxol monotherapy.

In this study, we investigated the expression of MDR-1 in Caki-2 RCC cell and

human RCC cell that was obtained from the patients with lymph nodes metastasis, then each combination effect of taxol,doxorubicin with 1B3PY1 in cultured Caki-2 cell and human RCC cell.

II. MATERIALS AND METHODS

1. Reagents

Caki-2 cell line (cell line organism: human, source organ: kidney, disease : clear cell carcinoma, morphology: epithelial) were obtained from ATCC (Rockville, MD). Clinical samples were obtained from 8 males and 2 females with conventional clear cell RCC who had metastatic lesions at operation. All patients were untreated by chemotherapy prior to the collection of samples. All specimens were obtained from primary RCC lesion immediately upon surgical resection, and contained in transport media.

2. Cell culture

After emzymatic dissociation of Caki-2 cell line, the cells were centrifuged at 800rpm for 5min and the pellet was resuspended in MEM medium supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, and a 2-fold vitamin solution . And then cells were seeded in 100mm cell culture dish. Tumor tissue was rinsed many times in cold (4°C) Eagle's minimum essential medium containing 1% bovine serum albumin and antibiotics [penicillin,

100 units/ml; streptomycin, 100μg/ml; amphotericin B, 3μg /ml; gentamicin, 50μg/ml]. The tissue was then cut into 1-3-mm^3 fragments using a sterile scalpel blade. The fragments were subjected to a sequential enzymatic digestion of 30 min at 37°C in Eagle's minimum essential medium containing collagenase type I (200units/ml) and DNase (270units/ml) .To complete the dissociation process, hyaluronidase type IV (35 NF units/ml; Sigma) was added to the enzyme mixture. After emzymatic dissociation, the cells were centrifuged at 800rpm for 5min and the pellet was resuspended in MEM medium supplemented with 10% fetal bovine serum, sodium pyruvate, nonessential amino acids, L-glutamine, and a 2-fold vitamin solution . And then cells were seeded in 100mm cell culture dish.

3. Expression of MDR-1 in Caki-2 cell and human RCC cell: RT-PCR

A PCR primer (MDR-1-F; gcctggcagctggaagacaaatacacaaaatt, MDR-1-R; cagacagcagctgacagtccaagaacaggact) was synthesized based on MDR1 gene code to estimate MDR1 in Caki-2 cell and human RCC cell. We extracted RNA by Tissue RNA PrepMate kit (Bioneer cop.), then reacted with reverse transcription kit (Accu

Power PCR Premix, Bioneer cop.) and estimated the degree of gene expression by PCR. GAPDH cDNA was used as a control.

4. Cytotoxicity of 1B3PY1 in Caki-2 cell and human RCC cell

We calculated the cell viability in the response to 1B3PY1 in both Caki-2 cell and human RCC cell by CCK assay (Cell counting kit-8, DOJINDO LAB.) XR9576 was used as a control.

5. Combination effect of taxol, doxorubicin with 1B3PY1 in Caki-2 cell

We observed the combination effect of taxol,doxorubicin with various concentration of 1B3PY1 (1uM, 3uM, 10uM) in cultured Caki-2 cell line.

In vitro chemosensitivity of the cell lines to the drugs was determined using a modified MTT assay. The assay was optimized for cell line where 5 days of cell growth after drug treatment were observed to be necessary to allow sufficient time for cell death and loss of metabolic activity to occur. Growth curves were obtained for each of the cell lines to determine appropriate seeding densities for each cell line into 96-well plates to ensure that the cells remained in exponential growth throughout the

entire assay. The IC_{50} is defined as the drug concentration that resulted in a 50% reduction in absorbance at 540 nm in drug-treated wells relative to control wells with the untreated controls being assigned a value of 100%.

6. Combination effect of taxol, doxorubicin with 1B3PY1 in human RCC cell

We observed the combination effect of taxol, doxorubicin with various concentration of 1B3PY1 (1uM, 3uM, 10uM) in cultured human RCC cell.

7. Data analysis

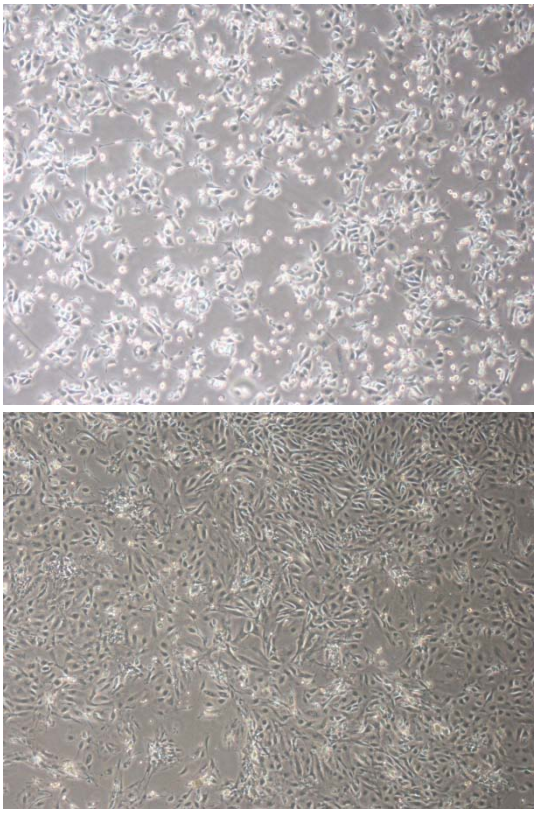
For data from the *in vitro* experiments, we performed statistical comparisons among groups using Student's t-test or the Mann-Whitney U test. Differences between groups were considered statistically significant at $P < 0.05$. The Statistical Package for Social Sciences (SPSS) for Windows, version 12.0, was used for statistical analysis. Data are expressed as mean ± standard deviation. All *in vitro* experiments were repeated with triplicate or quadruplicate samples and similar results were obtained across trials.

III. RESULTS

1. Primary cell culture

We cultured Caki-2 cell and human RCC cell (Fig.2).

Fig.2. Cultured Caki-2 cell and human RCC cell.

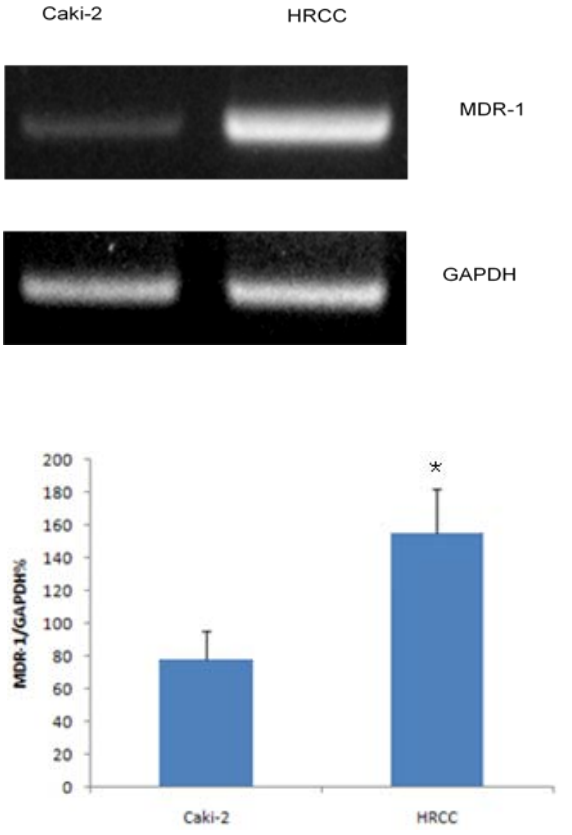


2. MDR1 expression in Caki-2 cell and human RCC cell

MDR1 was expressed in both Caki-2 cell and human RCC cell. The MDR1 in human

RCC cell was significantly higher than that of Caki-2 cell (Fig. 3).

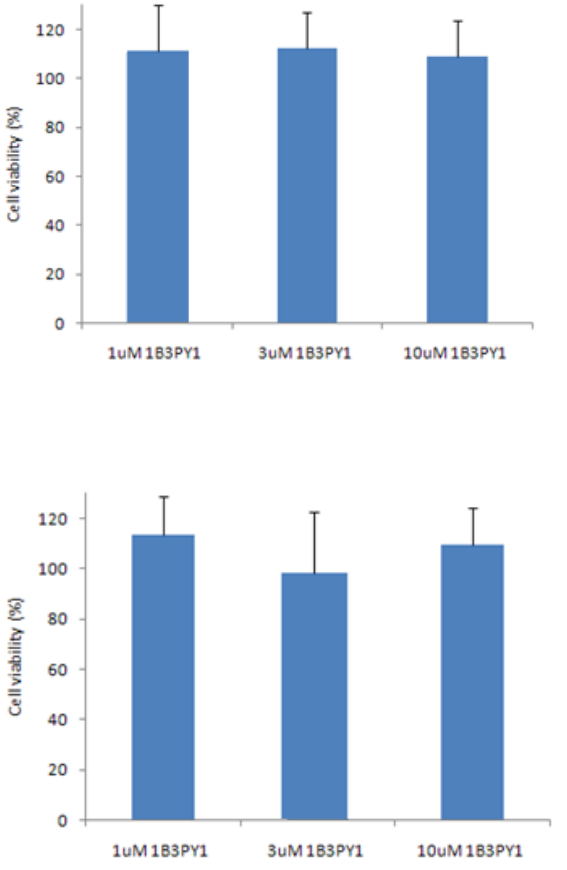
Fig. 3. MDR1 expression in Caki-2 cell and human RCC cell



3. Cytotoxicity of 1B3PY1 in Caki-2 cell and human RCC cell

Cell viability of Caki-2 cell and human RCC cell were not significantly changed when 1 uM, 3 uM, 10 uM 1B3PY1 were infused in each cell (Fig. 4).

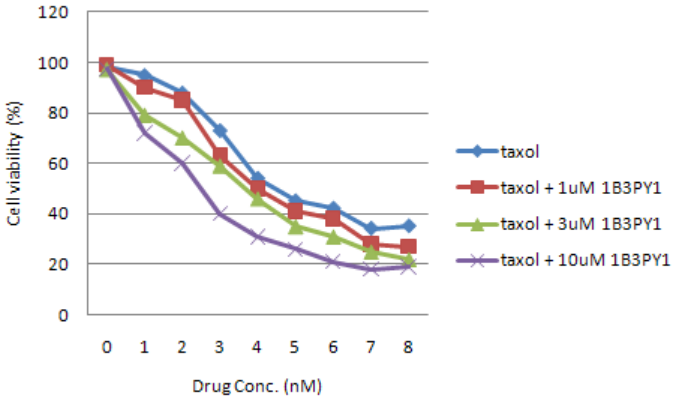
Fig. 4. Cell viability in Caki-2 cell and human RCC cell by 1B3PY1

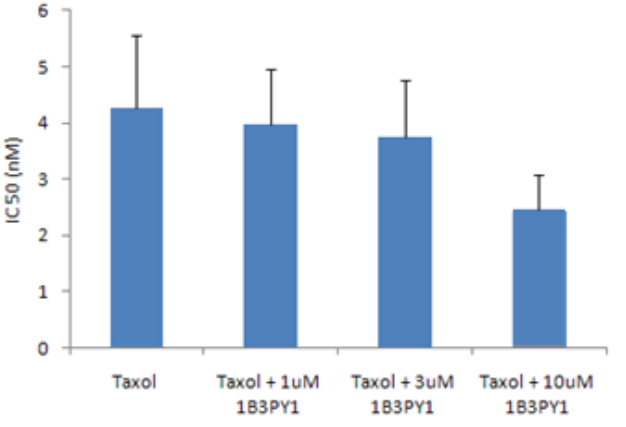


4. Combination effect in Caki-2 cell line with Taxol and 1B3PY1

Compared with IC_{50} of taxol only, there were no significant combination effect with taxol and 1B3PY1 (1uM, 3uM, 10uM) in Caki-2 cell (Fig. 5).

Fig. 5. Comparion of IC_{50} in Caki-2 cell between taxol and the combination of taxol and 1B3PY1



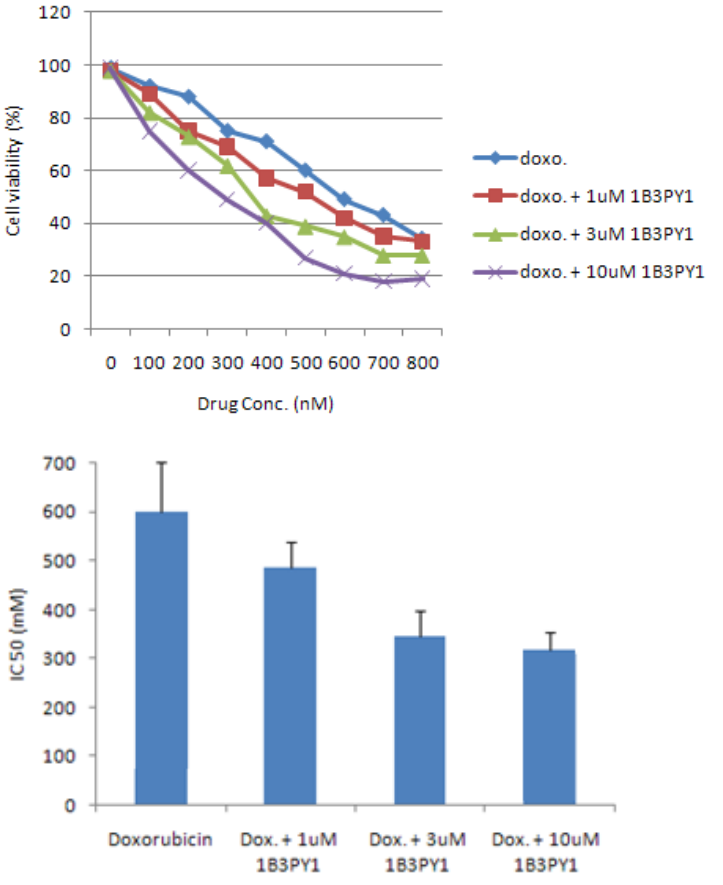


5. Combination effect in Caki-2 cell line with doxorubicin and 1B3PY1

Compared with IC_{50} of doxorubicin only, there were no significant combination

effect with doxorubicin and 1B3PY1 (1uM, 3uM, 10uM) in Caki-2 cell (Fig. 6).

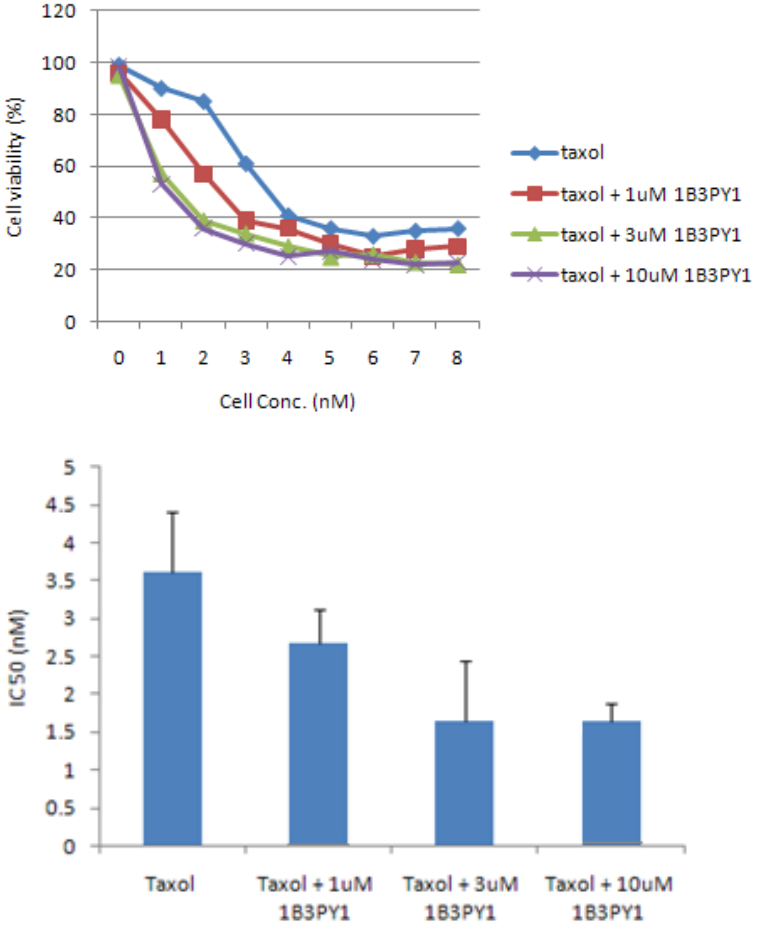
Fig. 6. Comparion of IC_{50} in Caki-2 cell between doxorubicin and the combination of doxorubicin and 1B3PY1



6. Combination effect in human RCC cell with taxol and 1B3PY1

Compared with IC_{50} of taxol only, there were no significant combination effect with taxol and 1B3PY1 (1uM, 3uM, 10uM) in human RCC cell (Fig. 7).

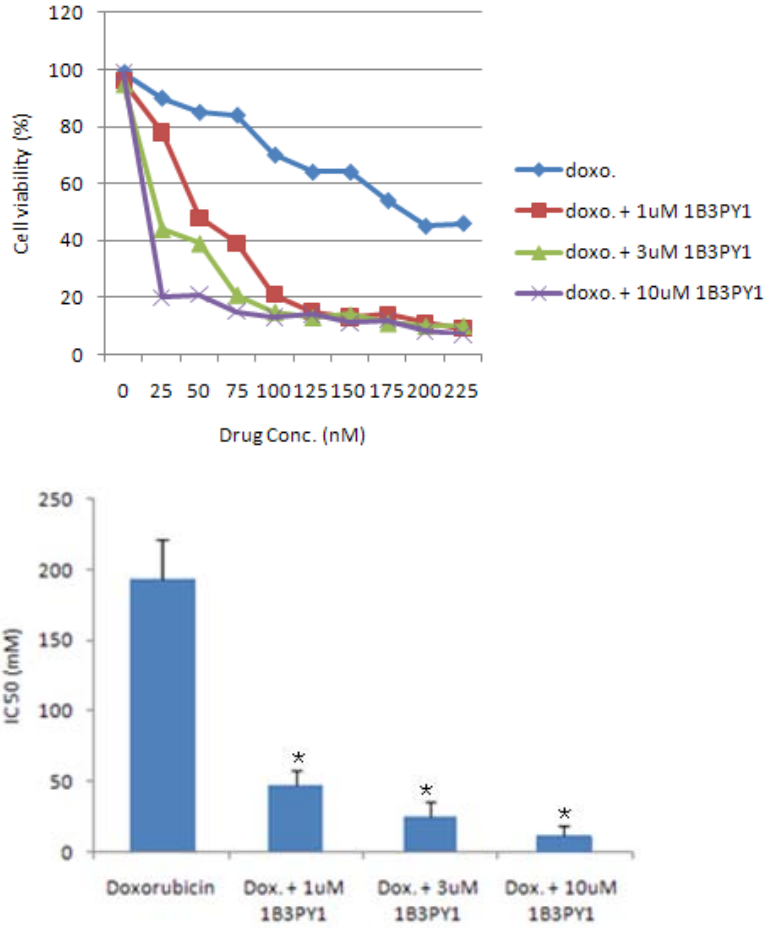
Fig. 7. Comparion of IC_{50} in human RCC cell between taxol and the combination of taxol and 1B3PY1



7. Combination effect in human RCC cell with doxorubicin and 1B3PY1

IC_{50} of doxorubicin and 1B3PY1 was significantly decreased compared with that of doxorubicin only with maximum of 19 times (Fig. 8).

Fig. 8. Comparion of IC_{50} in human RCC cell between taxol and the combination of doxorubicin and 1B3PY1



IV. DISCUSSION

RCC, which accounts for 2% to 3% of all adult malignant neoplasms, is the most lethal of the urologic cancers. Traditionally, more than 40% of patients with RCC have died of their cancer, in contrast with the 20% mortality rates associated with prostate and bladder carcinomas.[4,32] In Korea, 4,771 new cases of RCC were diagnosed from 2003 to 2005, which was 15th in all cancers. (National cancer cencer. www.ncc.re.kr) This is primarily a disease of the elderly patient, with typical presentation in the sixth and seventh decades of life.[4] Unfortunately, the incidence of renal cell carcinoma is gradually increasing, and despite a trend toward earlier detection, mortality rates remain high. The mortality rates from RCC would presumably be even higher if not for the trend toward incidental detection.[4,33-35]

1B3PY1 has the typical features of 3rd generation MDR1 inhibitor, ie, high affinity, specificity, and no substrate for MDR1.[36] In this study, we found in human RCC cell, there was no significant combination effect with taxol and 1B3PY1, whereas IC_{50} in group of doxorubicin and 1B3PY1 was significantly

decreased compared with that of doxorubicin monotherapy, maximum difference of which was upto 19 times. Why does such difference exist? Although MDR1 was expressed in Caki-2 cell and human RCC cell, the MDR1 in human RCC cell was significantly higher than that of Caki-2 cell.

Studies detailing the prevalence and contribution of MDR1 in RCC are conflicting. MDR1 expression has been reported widely in untreated RCC.[12,37] It does appear that intrinsic drug resistance exists in many RCC and it is associated, at least in part, with increased expression of MDR1. Evidence that chemoresistance in RCC is not exclusively determined by MDR1 is provided by the observations that some primary cell lines or cultures are resistant in the absence of detectable MDR1 expression and the resistance is not reversed by inhibitors of MDR1.[12,37-9] Clinical studies to date have failed to provide convincing evidence for MDR1-mediated resistance in RCC, with trials of MDR1 antagonists having limited success,[9] although this may be attributed to lack of potency and specificity of the early-generation antagonists.[40]

The resistance to therapy has been correlated to the presence of three

molecular “pumps: that actively expel chemotherapeutics out of tumor cells: P-glycoprotein (MDR1/Pgp), multidrug resistance-associated protein (MRP), and lung resistance-related protein (LRP; also referred to as major vault protein, MVP).[41,42] In this regard, the most typical efflux pump in the cell membrane is represented by MDR1 transporting various xenobiotics out of cells by using ATP.[11] MDR1 has been implicated in both intrinsic and acquired resistance in a number of cancers, including RCC, where MDR1 transcripts or protein have been identified in normal renal proximal tubules[43] and in the majority of RCC samples,[13,37,44-46] often at higher levels than other tumor types. Experimental data also supporting a role for MDR1 in RCC resistance include high MDR1 mRNA levels in several renal tumor cell lines examined as part of the National Cancer Institute (NCI) *in vitro* anticancer drug screen (http://dtp.nci.nih.gov),[47,48] and increased MDR1 mRNA levels associated with *in vitro* resistance of cell lines or primary cultures to paclitaxel[38] and doxorubicin.[49] However, in one study, MDR1 expression was detected in only 21% of RCC tissue samples.[11] Having outlined the difficulties in detecting

MDR1 in cancer cells, it is not surprising that our knowledge about the expression of MDR1 protein from sample to sample may be incomplete.

The nex efflux pump of the mammalian cell membrane is represented by MRP.[50] Two in particular, MRP1 (ABCC1) and the human canicular multispecific organic anion transporter (cMOAT, MRP2, ABCC2), have been shown to be overexpressed in cell lines that exhibit non-MDR1-mediated resistance.[51] MRP1 is expressed in most human tissues, whereas MRP2 has limited tissue expression but is expressed on the luminal membrane of proximal tubules in normal kidney.[52,53] MRP2 has been shown to modulate resistance to several chemotherapeutic agents *in vitro*, including vincristine,[54,55] and is expressed in RCC tissue.[52,53] However, the role of pan-MRP in RCC resistance has only been studied as part of the NCI anticancer screen and the contribution of individual members of this large family has not been examined.[56] These two mentioned resistant proteins belong to the ABC superfamily, which contributes to drug reistance via ATP hydrolysis. The structural similarity between MRP1 and MDR1 are parallel by an overlap in their drug resistance spectra, although

taxanes are a notable exception as they are poor substrates for MRP1. In normal renal tissue, MRP1 is localized in epithelial cells of proximal tubules. Hodorova et al. reported positive immunostaining of MRP1 was obwerved in 62% of RCC tissue samples.[11] Walsh et al. showed high levels of MRP-1 protein expression in RCC; all tumours investigated showed MRP-1 protein expression with 61% of tumours exhibiting MRP1 positivity in at least 50% of tumour cells.[2] The only paper demonstrating MRP2 expression in RCC was published by Schaub, who showed its expression in 95% of renal clear-cell patho-histology instances.[52] These observed high level of MRP protein expression suggests that this efflux pump also may be playing a contributing role in the chemoresistance of RCC.

In addition to ABC transporters, LRP, which is a major component of cytoplasmic vaults and may play a role in drug sequestration into exocytotic vesicles, has been implicated in drug resistance, being a superior predictor for *in vitro* resistance in 61 cancer cell lines overall compared with MDR1.[56,57] LRP is expressed in normal renal proximal and distal tubules and Bowman's capsule

and increased expression is seen in renal tumors.[58,59] LRP mRNA levels have been associated with the multidrug resistance phenotype in a panel of intrinsically resistant tumor cell lines, including an RCC line,[60] providing limited evidence for a potential role in chemotherapy resistance in RCC. The analysis of LRP expression in set of RCC revealed about 77% positivity in conventional as well as other type RCC. The total number of MDR1 positively staining tumors was much lower in comparison with MRP1 and LRP positive samples.[11]

In this study, we did not investigate the other efflux pumps including MRP1, 2, and LRP, therefore, it will influence to the response rate of specific chemoagents. The respective positive rates of each MDRs in RCC were somewhat variable. It will be important to modulate all MDRs in managing RCC. We did not evaluate the expression of MDR1 after combination of 1B3PY1 in Caki-2 and human RCC cell because 1B3PY1 temporarily blocked MDR1.

Despite more than 30 years of effort and numerous clinical trials, many

fundamental questions remain to be answered before modulation of multidrug resistance can become an effective clinical tool.[57] New MDR1 inhibitor, 1B3PY1 can give a new perspective in the treatment of metastatic RCC. Our findings may help to characterize pathologic mechanisms and to identify potential therapeutic strategies for RCC that deserves to be explored in depth.

V. CONCLUSIONS

MDR1 was expressed in Caki-2 cell line and human RCC cell. The MDR1 in human RCC cell was significantly higher than that of Caki-2 cell. New MDR1 inhibitor, 1B3PY1 revealed no cytotoxicity in Caki-2 cell and human RCC cell. Compared with IC_{50} of taxol or doxorubicin only, there were also no significant combination effect with taxol or doxorubicin and 1B3PY1 in Caki-2 cell. In human RCC cell, there was also no significant combination effect with taxol and 1B3PY1, whereas IC_{50} in group of doxorubicin and 1B3PY1 was significantly decreased compared with that of doxorubicin monotherapy, maximum difference of which was upto 19 times. As a new MDR1 inhibitor, we suggest IB3PY1 would give a perspective in the treatment of metastatic RCC. Further study will be needed.

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ABSTRACT

신세포암에서 다약제내성인자의 발현과 다약제내성인자 억제제 효과

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양 원 재

목적: 신세포암 (renal cell carcinoma: RCC)은 미국의 경우 모든 성인암의 2-3%를 차지하며 비뇨기계암 중 가장 치명적인 암이다. 다른 암과 비교해 봤을 때 RCC의 특징은 기능적 혹은 구조적으로 뚜렷한 유사성이 없는 다양한 형태의 여러 항암제에 높은 약제내성을 나타낸다는 것이다. 이러한 현상을 다제약제내성 (multidrug resistance: MDR)이라고 한다. 현재까지 항암요법을 대치할 수 있는 치료법이 적은 상태에서 진행된 RCC는 이러한 이유 때문에 나쁜

예후를 보일 수 밖에 없다. MDR1으로 불리기도 하는 P-glycoprotein은 RCC의 80-90%에서 발현되어 energy-dependent efflux pump로 작용하여 항암제와 같은 소수성 복합물을 세포 밖으로 배출시킨다. 따라서 진행된 RCC에서 이러한 MDR1을 억제할 수 있는 방법에 대한 연구는 매우 시급히 이루어져야 할 중요한 분야라고 여겨진다. 본 연구에서 사용할 MDR1 억제제는 1B3PY1 화합물이다. 이는 새로 개발된 MDR1 억제제로서 일차적으로 항암 내성 효과가 있는 화합물의 구조를 분석하여 MDR1을 억제할 수 있는 분자를 설계한 후 50여 가지의 유도체 화합물을 합성하고 MDR1을 가진 암세포를 대상으로 MDR1 억제 활성을 측정하였고 합성된 50 여개의 화합물 중에서 MDR1 억제 활성이 가장 좋은 것을 1B3PY1로 명명하였다. 이에 본 연구에서는 RCC 세포주인 Caki-2 cell line과 실제 림프절 전이를 보인 human RCC의 콩팥내 원발암의 RCC cell을 primary culture하고 각 cell 내의 MDR1 level과 1B3PY1 화합물 level을 RT-PCR로 확인하여 MDR1 level과 약효와의 관련성 및 약물자체의 cytotoxicity를 확인한 후, 1B3PY1 화합물과 항암제 (taxol, doxorubicin)를 동시에 투여한 군과 항암제만을 투여한 군에서 치료 효과가 어떤 차이를 보이는지를 비교해 보았다.

대상 및 방법: Caki-2 cell line과 human RCC cell의 1B3PY1 화합물

level을 RT-PCR로 확인하여 MDR1 level과 약효와의 관련성 및 약물자체의 cytotoxicity를 확인한다. Caki-2 cell과 실제 수술 당시 림프절 전이를 보인 10명의 전이 clear cell type RCC 환자의 신장내 원발 병소를 술장에서 바로 처리하여 각각 배양한다. 두 군에서 taxol, doxorubicin과 여러 농도의 1B3PY1 화합물 1uM, 3uM, 10uM을 병용 투여한 군과 항암제만을 투여한 군에서의 치료 효과를 비교한다. 결과: Caki-2 cell에 대해 각각 1, 3, 10uM 1B3PY1을 처리시 cell viability는 각각 110.4, 112.3, 108.7%로 1B3PY1의 cytotoxicity는 거의 없었다. Human RCC cell에 각각 1, 3, 10uM 1B3PY1을 처리시 cell viability는 각각 113.7, 98.3, 109.3%로 1B3PY1의 cytotoxicity는 거의 없었다. RT-PCR을 통해 Caki-2와 human RCC cell의 MDR1 유전자의 발현을 확인한 결과 양 군에서 모두 발현되었고 환자 RCC cell 에서 통계적으로 의미있게 많이 발현되었다.

배양된 Caki-2 cell에 taxol, taxol+1uM 1B3PY1, taxol+3uM 1B3PY1, taxol+10uM 1B3PY1의 IC_{50}은 각각 4.25, 3.96, 3.75, 2.44nM로 taxol만을 처리했을 때와 1B3PY1을 동시에 처리했을 때를 비교시 IC_{50}의 차이는 미미하였다. Doxorubicin, doxorubicin+1uM 1B3PY1, doxorubicin+3uM 1B3PY1, doxorubicin+10uM 1B3PY1의 IC_{50}은

각각 602.6, 485.7, 345.3, 307.9nM로 doxorubicin만을 처리했을 때와 1B3PY1을 동시에 처리했을 때를 비교시 1B3PY1를 처리했을 때 IC_{50}가 약 2배 정도 감소하는 효과를 확인하였다.

환자에서 배양된 RCC cell에 Taxol, taxol+1uM 1B3PY1, taxol+3uM 1B3PY1, taxol+10uM 1B3PY1의 IC_{50}은 각각 3.62, 2.39, 1.65, 1.45nM로 taxol만을 처리했을 때보다 1B3PY1을 동시에 처리했을 때 IC_{50}의 차이는 미미하였다. Doxorubicin, doxorubicin+1uM 1B3PY1, doxorubicin+3uM 1B3PY1, doxorubicin+10uM 1B3PY1의 IC_{50}은 각각 193.1, 47.5, 25.5, 11nM로 doxorubicin만을 처리했을 때보다 1B3PY1을 동시에 처리했을 때 IC_{50}가 약 19배 정도 감소하는 효과를 확인하였다.

결론: 기존에 알려진대로 Caki-2 cell과 human RCC cell에서 MDR1이 발현되었다. 새로이 합성된 MDR1 inhibitor인 1B3PY1는 Caki-2 cell과 human RCC cell에 대한 cytotoxicity를 보이지 않았다. Caki-2 cell culture 후 taxol만을 처리했을 때와 1B3PY1을 동시에 처리했을 때를 비교시 IC_{50}의 차이는 미미하였고, doxorubicin만을 처리했을 때와 1B3PY1을 동시에 처리했을 때를 비교시 1B3PY1를 동시에 처리했을 때 IC_{50}가 약 2배 정도 감소하는 효과를 확인하였다. 림프절 전이가 있었던 human RCC의 신장내 원발암 조직을 대상으로

human RCC cell culture 후의 같은 방법의 실험에서 taxol과 taxol+1B3PY1 처리군의 IC_{50}의 차이는 미미한 반면 doxorubicin만을 처리했을 때보다 1B3PY1을 동시에 처리했을 때 IC_{50}가 약 19배 정도 감소하는 효과를 확인하였다.새롭게 개발된 MDR1 억제제인 1B3PY1은 RCC의 항암치료 효과를 높일 수 있는 가능성을 보이나 실제 임상에 적용되기까지 더 많은 연구가 필요하다.

핵심되는 말: 신세포암, 다약제내성, 항암치료, taxol, doxorubicin