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คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น

Antagonistic Growth Inhibitory Effect of Isomorellinol with Chemotherapeutic Agents Against Human Cholangiocarcinoma KKU-M156 Cell Line

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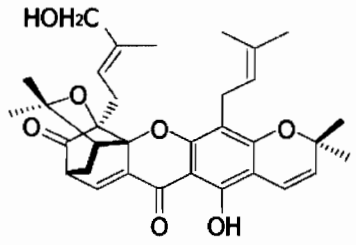
Introduction

Cholangiocarcinoma (CCA) is a malignant tumor arising from bile duct epithelial cells, characterized by a poor prognosis and unresponsive to conventional chemotherapeutic agents[1]. Our previous studies demonstrated that isomorellinol (Figure 1) inhibited growth, induced cell cycle arrest and apoptosis in CCA cell lines[2]. This study aims to examine the growth inhibitory effects of isomorellinol, 5-Fluorouracil (5-FU) and paclitaxel and their combination effects on human CCA KKU-M156 cell line.



Isomorellinol

Figure 1 Chemical structure of isomorellinol.

Materials and Methods

Chemicals

RPMI 1640 medium, fetal bovine serum (FBS), Fungizone, Penicillin-Streptomycin were purchased from Gibco (Rockville, MD, USA). 5-FU and paclitaxel were obtained from Boryung Pharmaceutic Co. LTD (Korea). Isomorellinol was isolated from Garcinia hanburyi Hook.f. (family Guttiferae) using bioassay-directed fractionation. All other chemicals were analytical grade.

Human CCA cell line

The human CCA cell line, KKU-M156 was established from CCA patients in the Faculty of Medicine, Khon Kaen University, Thailand. Cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin and 100 µg/ml streptomycin at 37 °C in a 5% CO_2 incubator.

Cell growth inhibition assay

The sulforhodamine B (SRB) assay was performed to assess growth inhibition using a colorimetric assay, which estimates cell number indirectly by staining total cellular protein with the dye SRB[3]. Briefly, human CCA KKU-M156 cell ($1x10^4$ cells/well) was seeded in 96-well microtiter plates and incubated at 37°C for 24 h to allow for cell attachment. Cells were treated with 0, 0.5, 1, 2, 4 and 8 µM/well for isomorellinol; 0, 4, 8, 16, 32 and 64 µM/well for 5-FU; and 0, 0.01, 0.02, 0.04 and 0.08 µM/well for paclitaxel. The plates were incubated for 1 h (d0) and 72 h (d3) at 37 °C . At the end of each exposure time, the medium was removed and the cells were fixed with 20% (w/v) trichloroacetic acid

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at 4°C for 1 h, stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid for 30 min, and washed four times with 1% acetic acid. The protein-bound dye was solubilized with 10 mM Tris base (pH 10). The absorbance (OD) was measured using a microplate reader (Sunrise-TECAN, USA) at 510 nm. Percentage of cell survival was calculated using the formula: Percentage of cell survival = [(OD test sample at d 3 - OD d 0)/(OD control at d 3 - OD d 0)] x 100. Dose-response curves were plotted, and 50% growth inhibitory concentrations of isomorellinol or drugs (IC_{50}) were calculated through computation with the CalcuSyn software program (Biosoft, Cambridge, UK).

Evaluation of drug interaction

Combination assays were performed using appropriate concentrations of isomorellinol with appropriate concentrations of 5-FU or paclitaxel. Cells treated with the same final concentrations of the isomorellinol or chemotherapeutic drugs alone were also examined. Cell growth inhibition was determined using the SRB assay, as previously described. In the assessment of the combination effects (i.e., synergism, additivity or antagonism), the combination index (CI) method according to Chou and Talalay was used[4].

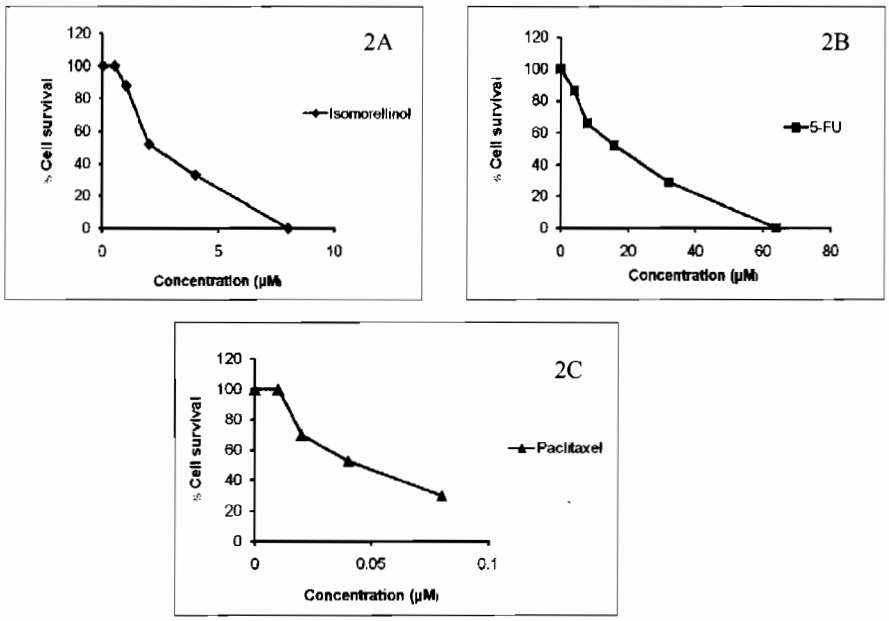
Statistical analysis

Data were expressed as mean ± standard errors. Comparisons between untreated control cells and the treated cells were made using pair *t*-test. Differences were considered significant at $^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$. All analyses were performed using SPSS version 10.0 (spss Inc, USA).

Results

Effect of isomorellinol, 5-FU and paclitaxel alone on KKU-M156 cell growth

The treatment of KKU-M156 cell with isomorellinol, 5-FU and paclitaxel markedly inhibited cell growth in a dose-dependent manner (Figure 2). The IC50 values of isomorellinol and these chemotherapeutic drugs are shown in Table 1. The anticancer potency of isomorellinol is comparable to these two drugs. These results demonstrated the efficacy of isomorellinol and these drugs in KKU-M156 cell growth inhibition.



Fiugre 2 Growth inhibitory effects of isomorellinol (2A), 5-FU (2B) and paclitaxel (2C) on KKU-M156 cell. Cells were treated with DMSO or indicated amount of isomorellinol and these chemotherapeutic drugs for 72 h. Cell viability was determined by SRB assay. Each value represents the mean±S.E. of three independent experiments.

Table 1 The IC_{50} values of the Isomorellinol and chemotherapeutic drugs on CCA cell lines.

Drug	IC_{50} value (?M)
Isomorellinol	2.4 ± 0.05
5-FU	10.03 ± 0.16
Paclitaxel	0.045 ± 0.40

Combination effects of isomorellinol with chemotherapeutic drugs on cell growth

Based on the data showing efficacy of isomorellinol, 5-FU and paclitaxel in KKU-M156 cell, next we assessed their effects in combinations on KKU-M156 cell growth and analyzed the data for a possible synergism (CI<1), additivity (CI=1) or antagonism (CI>1) by using median effect analysis. As shown in Figure 3, the combinations of isomorellinol with both drugs resulted in antagonistic effects in all combinations tested.

Discussion

Because of the simple, flexible, rapid and economic features, investigations of drug combinations *in vitro* can frequently be used for prospective or retrospective studies in animal systems or in clinical settings. *In vitro* studies may also provide the rationale for drug combinations based on quantitation of synergism or antagonism. In this study, by using the median-effect principle and the combination index-isobologram technique, we were capable of analyzing the growth inhibitory effects of isomorellinol, 5-FU or paclitaxel used alone or in combinations. Our results demonstrated that isomorellinol and both chemotherapeutic drugs mediated significant growth inhibitory effects on KKU-M156 cell in a dose-dependent manner. Isomorellinol combined with 5-FU or paclitaxel resulted in antagonistically growth inhibitory activity at all concentrations tested in KKU-M156 cell.

In previous reports, 5-FU was found to inhibit DNA and RNA synthesis of cell which usually occur during S phase of cell cycle. Whereas, paclitaxel was found to interfere with normal function of microtubule break down leading to cell mitotic inhibition. Previously, we found that isomorellinol induced cell cycle arrest at G0/G1 phase in KKU-M156 cells (data not shown). Therefore, the antagonistic effects of the combinations may be due to the G0/G1 phase arrest of cell cycle caused by isomorellinol leading to decrease number of cell in S- and G2- phase which are target for 5-FU and paclitaxel, respectively.

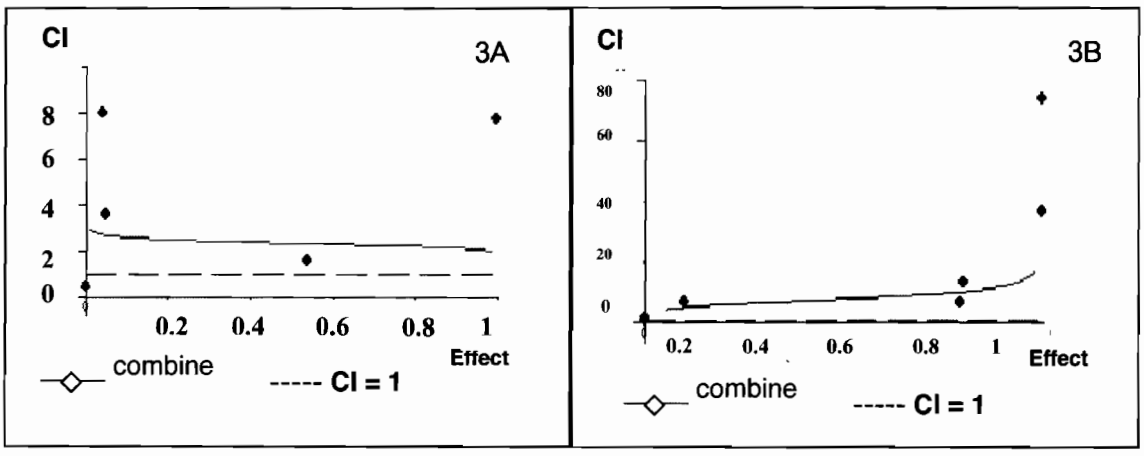


Figure 3 The combination effects of isomorellinol with 5-FU or paclitaxel on KKU-M156 (3A, 3B). Curve with solid lines are computer simulated fa-CI plots, base on the parameters (m and Dm values) for the combination of isomorellinol with 5-FU or paclitaxel.

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Conclusion

The growth inhibitory activity of 5-FU or paclitaxel, as a single agent, may be reduced by combination with isomorellinol.

Acknowledgments

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