Antagonistic Growth Inhibitory Effect of Caged Xanthones with Chemotherapeutic Agents against Human Cholangiocarcinoma KKU-M214 Cell Line

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Background and Objective : Cholangiocarcinoma (CCA) is a malignant tumor of cholangiocytes, characterized by a poor prognosis and poor response to conventional chemotherapeutic agents. Our previous studies have demonstrated that isomorellin and isomorellinol inhibited cell growth, induced cell cycle arrest and apoptosis in KKU-100 and KKU-M156 cell lines. The aim of this study is to examine the growth inhibitory effects of isomorellin, isomorellinol and cisplatin or their combinations on human KKU-M214 cell line.

Methods : KKU-M214 cells were treated with isomorellin, isomorellinol and cisplatin or their combinations and cell viability was determined using sulforhodamine B (SRB) assay. The interactions between isomorellin or isomorellinol with cisplatin were analyzed by isobologram and combination index (CI) method of Chou-Talalay.

Results : Concentration of isomorellin, isomorellinol and cisplatin were found to inhibit growth of KKU-M214 cell line in a dose-dependent manner with the IC_{50} values of 0.61 ± 0.02, 1.12 ± 0.02 and 17.16 ± 0.06 μM, respectively. The combinations of isomorellin/cisplatin and isomorellinol/cisplatin showed antagonistic effect (CI > 1).

Conclusion: The combination of isomorellin or isomorellinol with cisplatin showed an antagonistic effect on KKU-M214 cells.

Key words: cholangiocarcinoma, isomorellin, isomorellinol, cisplatin, antagonistic effect

Proceeding

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Introduction

Cholangiocarcinoma (CCA) is a malignant cancer of cholangiocytes, characterized by a poor prognosis and poor response to conventional chemotherapeutic agents[1]. Our previous studies have been demonstrated that isomorellin and isomorellinol (Fig. 1) inhibited cell growth, induced cell cycle arrest and apoptosis in CCA KKU-M100 and KKU-M156 cell lines[2]. The aim of this study is to examine the growth inhibitory effects of isomorellin, isomorellinol and cisplatin or their combination effects on human CCA KKU-M214 cell line.

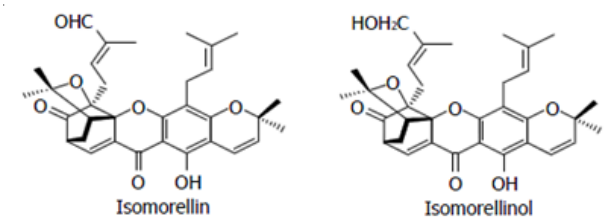

Figure 1 Chemical structure of isomorellin and isomorellinol.

Materials and methods

Chemicals

RPMI-1640 medium, fetal bovine serum (FBS) and Penicillin-Streptomycin were purchased from Gibco (Rockville, MD, USA). Cisplatin was kindly provided from Boryung Pharmaceutic Co. LTD (Korea). All other chemicals were analytical grade.

Human cancer call line

The human CCA cell line, KKU-M214 was established at the Department of Pathology, Faculty of Medicine, Khon Kaen University, Thailand. Cell was grown in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 100 μg/ml streptomycin and 100 U/ml penicillin in a humidified environment with 5% CO_2 at 37°C.

Plant compounds

Isomorellin and isomorellinol were isolated from *Garcinia hunburyi* Hook.f. (family Guttiferae) using bioassay directed fractionation by Professor Vichai Reutrakul, Department of Chemistry, Faculty of Science, Mahidol University, Thailand.

Preparation of plant compounds

Stock solution of each compounds were prepared by solubilizing with dimethylsulfoxide (DMSO) and was further diluted with culture media to yield the desired final concentration.

Preparation of chemotherapeutic drug

Cisplatin was dissolved with sterile normal saline (NSS) for injection at concentration of 2,000 mM. Serial dilutions of cisplatin were performed in culture medium, immediately before use, to obtain the required final concentrations.

Cell proliferation assay

The sulforhodamine B (SRB) assay is used in this study to estimate cell number indirectly by staining total cellular protein with the SRB. The protocol is based on that originally described by Skehan *et al.* (1990) [3] with some modifications. Briefly, 10^4cells/ml were seeded in 96-well microtiter plates and incubated for 24 hrs. After treatment for 72 hrs with 0.1375, 0.275, 0.55, 1.1, 2.2 and 4.4 μM/well for isomorellin; 0.075, 0.15, 0.3, 0.6, 1.2 and 2.4 μM/well for isomorellinol and 8.3, 9.9, 11.9, 14.3, 17.2 and 20.6 μM/well for cisplatin as a reference compound and DMSO as the solvent-control. Then the medium was removed and the cells were fixed with 10% (w/v) cold trichloroacetic acid (Metcalfe, Monson, deAllie, & Cohen), at 4°C for 1 hr. After washing with distilled water, cells were stained for 1 hr with 0.4% (w/v) SRB. After washing with 1% acetic acid bound dye was dissolved with 200 μl of 10 mM Tris buffer (pH 10) was recorded at 540 nm was recorded. Percentage of cell growth will be calculated using equation below. IC_{50} value was expressed as concentration of compounds in micromolar that caused a 50% growth inhibition comparing with controls.

$$\% \text{ Cell survival} = \left(\frac{\text{OD540 treated cells on day3 - OD 540 starting cells}}{\text{OD540 control on day3 - OD 540 starting cells}}\right) \times 100$$

Evaluation of drug interaction

Combination assays was performed using appropriate concentrations of isomorellin and isomorellinol with appropriate concentrations of cisplatin. Cells were treated with the same final concentrations of the compounds or cisplatin alone were also examined. Cell growth inhibition was determined using the SRB assay, as previously described. In the assessment of the combination effect, the combination index (CI) method of Chou and Talalay was used. The combination index (CI) will be calculated using equation below ((D)1 : Dose of cisplatin in combination to produce a median effect, (Dx)1 : Dose of cisplatin alone require to produce a median effect, (D)2 : Dose of compound in combination to produce a median effect and (Dx)2 : Dose of compound alone require to produce a median effect). Which the CI < 1 indicates synergism; CI = 1 indicates an additive effect; and CI > 1 indicates antagonism.

$$CI = \frac{(D)1}{(Dx)1} + \frac{(D)2}{(Dx)2}$$

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Comparisons between untreated control cells and treated cells were made using Student's *t*-test. Differences were considered significant at $p < 0.05$, $pb < 0.01$ and $pc < 0.001$. All analyses were performed using Microsoft Excel computer software (Microsoft, Redmond, WA, USA).

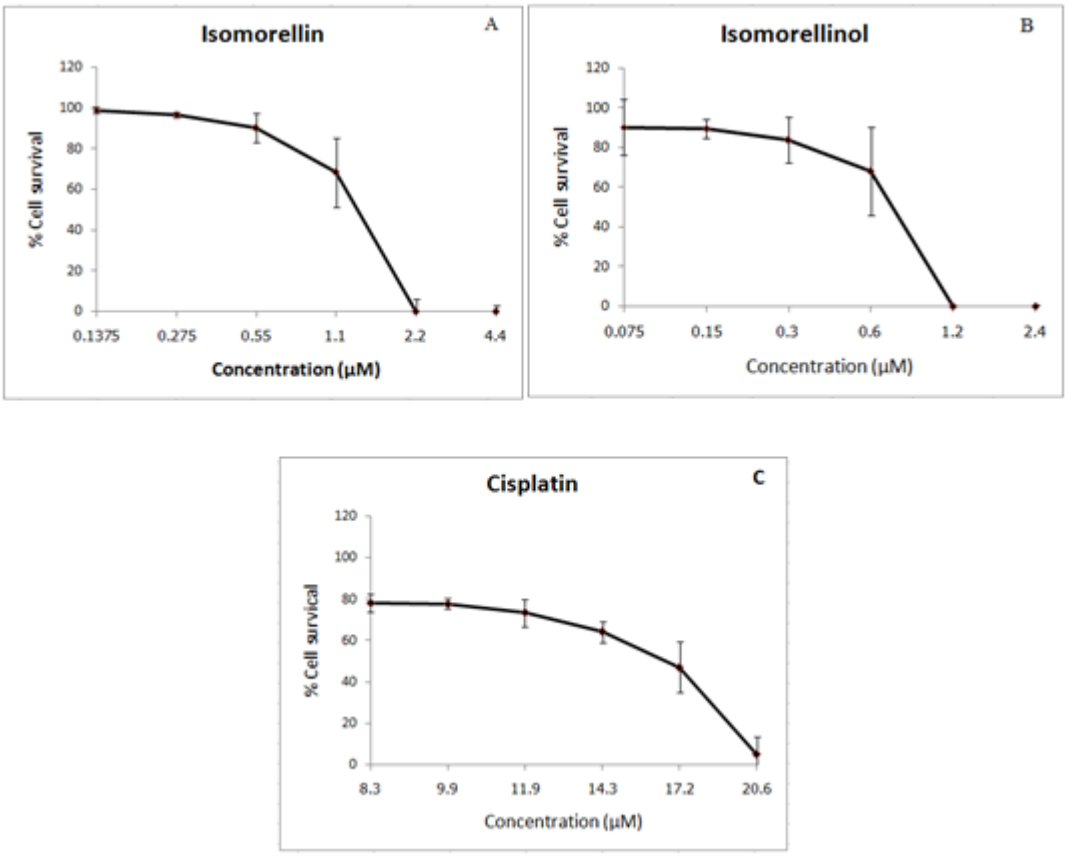

Figure 2 Growth inhibitory effects of isomorellin (A), isomorellinol (B) and cisplatin (C) on KKU-M214 cell. Cells were treated with DMSO or indicated amount of isomorellin, isomorellinol and cisplatin for 72 hrs. The percent cell viability was determined by SRB assay. Data are expressed as mean ± SD (n = 3).

Proceeding

Results

Growth inhibitory effects of isomorellin, isomorellinol and cisplatin on CCA KKU-M214 cell line.

Cytotoxic effect of isomorellin, isomorellinol and cisplatin on KKU-M214 was performed using SRB assay. Growth of KKU-M214 cell treated with isomorellin, isomorellinol and cisplatin was markedly inhibited in a dose-dependent manner (Fig. 2). The IC_{50} values of these two compounds and cisplatin are shown in Table 1. The anticancer potency of isomorellin and isomorellinol are better than cisplatin. These results demonstrated the efficacy of isomorellin, isomorellinol and cisplatin in KKU-M214 cell growth inhibition.

Combination effects of isomorellin and isomorellinol with cisplatin on KKU-M214 cell line

To determine the combination effect of isomorellin, isomorellinol and cisplatin on KKU-M214 cell by using SRB assay. The results were analyzed according to the method of Chou and Talalay[4]. The combinations of isomorellin/cisplatin and isomorellinol/cisplatin showed antagonistic growth inhibitory effect in KKU-M214 cells with CI > 1 (Fig. 3).

Discussion

Chemotherapy is an impotent issue in cancer, especially in CCA. However, the chemotherapy are

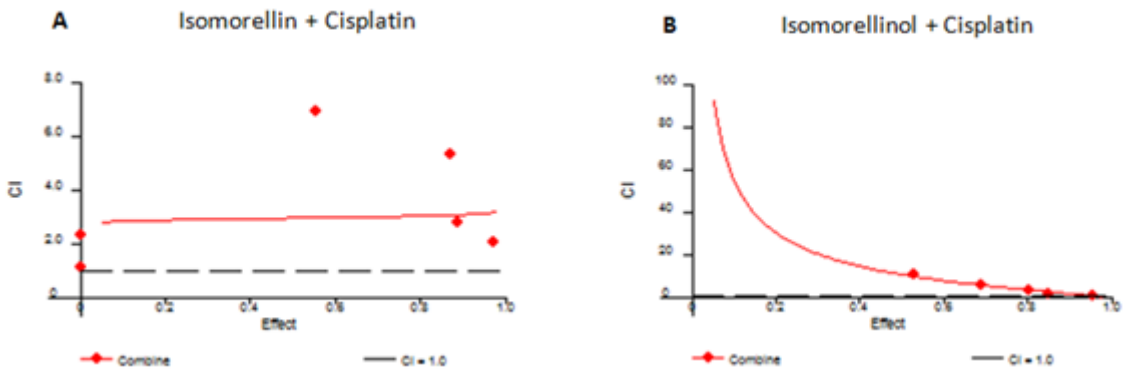

Figure3 Isomorellin, isomorellinol and cisplatin combination toxicity. KKU-M214 cells were treated with the appropriate concentrations of isomorellin and cisplatin or isomorellinol and cisplatin for 72 hrs. Combination index (CI) *vs* fraction affected (*fa*) plots obtained from the median-effect analysis program (Calcusyn, Biosoft, Cambridge, UK). Dashed line indicates a CI of 1. Each value represents mean of 3 independent experiments.

Table 1 Concentrations of isomorellin, isomorellinol and cisplatin causing a 50% inhibitory effect (IC_{50}) in KKU-M214 cell proliferation.

Compounds/Drug	IC_{50} values (μM)
Isomorellin	0.61 ± 0.02
Isomorellinol	1.12 ± 0.02
Cisplatin	17.16 ± 0.06

Data are mean ± SD of three independent experiments.

limited to use for treatment of cancer due to drug resistant and severe side effects. Accordingly, the combination of chemotherapeutic drugs and natural compounds with different molecular mechanisms are considered a promising therapeutic strategy with higher clinical efficacy. In this study, we investigated the combination effects of isomorellin and isomorellinol with cisplatin on KKU-M214 cell using the median-effect equation and CI method. Chemotherapeutic drugs indirectly induce apoptosis through DNA damage and cell cycle arrest. Cisplatin induces cell apoptosis by binding and causing crosslinking of DNA.

Previously, we found that isomorellin and isomorellinol induced apoptosis and induced cell cycle arrest at G0/G1 phase in KKU-M156 cells (data not shown)[5, 6]. Therefore, the antagonistic effects of the combinations may be due to the G0/G1 phase arrest of cell cycle caused by isomorellin or isomorellinol leading to decrease number of cell in S- and G2- phase which are target for cisplatin.

Conclusion

From our investigation, we concluded that isomorellin, isomorellinol and cisplatin or their combination has antagonistic effect in CCA KKU-214 cell line.

Acknowledgments

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