Comparison of in Vitro Cytotoxicity of Generic Paclitaxel and Irinotecan Formulations with their Reference Formulations on Seven Human Intrahepatic Cholangiocarcinoma Cell Lines

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BACKGROUND: Several chemotherapies have now been introduced for the treatment of cholangiocarcinoma (CCA). Although it has previously been reported that two new chemotherapeutic agents, paclitaxel and irinotecan, showed strong cytotoxic effect to human CCA cell lines the treatment cost currently using reference formulations of these two drugs are very expensive. To date, the generic drugs for both paclitaxel and irinotecan are now commercially available in Thailand with relatively low price compared to reference formulations. However, the study demonstrating the efficacy of the innovator and generic formulation of these two agents has never been reported.

OBJECTIVE: To determine and compare the cytotoxic activity of generic paclitaxel and irinotecan formulations with the reference formulations on seven human intrahepatic CCA cell lines.

DESIGN: in vitro study

SETTING: Faculty of Medicine, Khon Kaen University

MATERIAL AND METHOD: Cytotoxic activity of chemotherapeutic agent on CCA cell lines was determined by sulforhodamine B (SRB) assay. The IC50 value expressed as the concentration of drug that caused a 50% growth inhibition comparing with no drug treated control.
Introduction

Cholangiocarcinoma (CCA) is a bile duct tumor that is increasing worldwide in incidence and mortality. Evidence from clinical studies have demonstrated that response of this cancer to conventional chemotherapy is relatively poor. Nevertheless, 5-FU is a standard chemotherapy for CCA patients, only partial response to this drug is reported. Our recent in vitro study has shown that cytotoxicities of paclitaxel and irinotecan towards five human intrahepatic CCA cell lines isolated from Thai patients are very potent. In addition, clinical study suggest that combined regimen of paclitaxel and irinotecan can prolong stabilization of disease for more than 6 months in advanced cancer patients. This evidence suggests that paclitaxel and irinotecan may be useful for treatment of CCA patients. Both reference and generic formations of paclitaxel and irinotecan are now commercially available in Thailand. Although reference and generic formulations are pharmaceutically equivalent, there is no data available for either in vitro or in vivo to ensure that the efficacy of these formulations is similar. These data are necessary for physicians and pharmacists when prescribing or selecting the chemotherapy for their patients especially when cost-effectiveness is a great concern.

Cytoxic activity of the generic formulation was compared to the reference formulation using Student's unpaired t-test.

Results: Paclitaxel exhibited strong potency towards most CCA cell lines with IC\textsubscript{50} values ranging from 0.001-1.40 \(\mu\)M whereas irinotecan showed IC\textsubscript{50} values ranging varied from 0.02 to 69 \(\mu\)M. KKU-M055 was the most sensitive cell line to paclitaxel and KKU-OCA17 showed the highest sensitivity to irinotecan whereas KKU-M156 was the least sensitive cell line to both drugs. IC\textsubscript{50} values of the generic product (Intaxel\texttrademark, Dabur, India) and reference product (Taxol\texttrademark, Bristol-Myers Squibb, USA) formulations were not significantly different (\(P > 0.05\)). Similarly, the cytotoxicity of the generic formulation of irinotecan (Irinotel\texttrademark, Dabur, India) was not statistically different from the reference formulation (Campto\texttrademark, Aventis Pharma, UK).

Conclusion: The cytotoxic activity of paclitaxel towards six CCA cell lines was more potent than irinotecan except for KKU-OCA17. No different in the IC\textsubscript{50} values of the generic and reference formulations of paclitaxel and irinotecan against seven CCA lines suggest that the in vitro efficacy of generic and reference formulations of these two drugs are very similar.

Keywords: Cytotoxic activity, paclitaxel, irinotecan, cholangiocarcinoma, generic formulation

Objective

To compare the cytotoxic activity of the generic paclitaxel and irinotecan formulations to those of reference formulations on 7 different histological types of CCA cell lines.

Materials and Methods

Chemicals

Generic formulations of paclitaxel (Intaxel®, batch no. 5AH03, mfg date 01/2005, exp. date 12/2006) and irinotecan (Irinotel®, batch no. 5JL02, mfg date 04/2005, exp. date 03/2007) were from Dabur, Pharma Ltd., India. The reference formulation of paclitaxel (Taxol®) was from Bristol-Myers Squibb Company, USA (batch no. 5E07393, mfg date 18-05-05, exp date: 31-05-07) and the reference formulation of irinotecan (Campto®) was from Aventis Pharma, UK (Batch no. D4C271, mfg date 10/09/2004, exp date: 10/09/2007).

Ham’s F12, penicillin, streptomycin and trypsin-EDTA were purchased from Invitrogen Co., California, USA, fetal bovine serum from Seromed, Germany, and sulforhodamine B (SRB) from Sigma Chemical Co., USA. Tissue culture plates were obtained from Nunc, Denmark. All other chemicals were analytical grade.

Human CCA cell lines

Seven human intrahepatic CCA cell lines, namely, KKU-100 (poorly differentiated adenocarcinoma), KKU-M055 and KKU-M156 (moderately differentiated adenocarcinoma), KKU-M139 (squamous carcinoma), KKU-M213 (adenosquamous), KKU-M214 (moderately to poorly differentiated adenocarcinoma) and KKU-OCA17 (well differentiated adenocarcinoma) were used in this study. All cell lines were established in our Institute from CCA patients residing in northeastern Thailand. Cells were cultured in Ham’s F12 supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml of penicillin and 100 mg/ml of streptomycin at 37 °C with 5% CO2. The presence of mycoplasma contamination was periodically examined. These cell lines have been maintained in the laboratory for more than 6 years.

Cytotoxicity activity assay

Sulforhodamine B (SRB) assay was used to determine growth inhibition as described previously with some minor modifications. In brief, CCA cell lines (4 x 10^4 cells/ml) at exponential growth phase were trypsinized with 0.25% (v/v) trypsin and seeded in triplicate in 96-well flat-bottom microtiter plates and incubated for 24 h at 37 °C in a humidified 5% CO2 atmosphere. Then 100 µl aliquot of medium containing drug (from 0.0003 µg/ml to 300 µg/ml) or no drug as control was added to the 96-well plates and incubated at 37 °C for 72 h. The culture medium was subsequently removed and 200 µl aliquot of 10% (w/v) ice-cold TCA was added to each culture well. The plates were then incubated at 4 °C for 60 min. TCA-treated cells were stained for 30 min with 0.4% (w/v) SRB in 1% (v/v) acetic acid for 30 min, and subsequently washed five times with 1% (v/v) acetic acid to remove unbound stain. The plates were left to dry and the protein-bound stain was solubilized with 200 µl of 10 mM Tris base (pH 10.5) for 60 min. Absorbance was measured at 540 nm using a microplate reader (Tecan Austria GmbH, Austria). The concentration of drug required to inhibit cell proliferation by 50% (IC50) was determined by plotting the percentage of cell growth inhibition versus the drug concentration.

Statistical analysis

Tests were performed in 3 independent experiments. Data are expressed as mean % growth inhibition +SD. IC50 value represented the concentration of drugs that inhibited 50% cell growth. Comparison of IC50 values of generic formulation with reference formulation was analyzed using Student’s unpaired t-test.

Results

The cytotoxicity activities of the generic formulations of paclitaxel and irinotecan and their respective reference formulations, Taxol® and Campto® against the 7 CCA cell lines were shown in Table 1. There was not statistically significant difference in the IC50 values observed for generic and reference formulations of both paclitaxel and irinotecan. Moreover, results obtained from 7 CCA cell lines with different histological type clearly showed that all cell lines were sensitive to paclitaxel with IC50 values ranging from 0.001-1.40 µM. Among these cell lines, KKU-M055 and KKU-M214 showed very high sensitivity to paclitaxel with IC50 value less than 0.01 µM whereas KKU-M156 was the least sensitive (IC50 values of 0.6 and 1.4 µM). In the case of irinotecan, the IC50 values observed for the 7 CCA cell lines were in the range...
Table 1 Cytotoxic activities of Test formulations and Reference formulations on CCA cell lines. Data represents as mean IC₅₀ ± SD of at least three experiments.

<table>
<thead>
<tr>
<th>CCA Cell line</th>
<th>Histological type</th>
<th>Mean IC₅₀ (µM) of paclitaxel</th>
<th>Mean IC₅₀ (µM) of irinotecan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intaxel®</td>
<td>Taxol®</td>
</tr>
<tr>
<td>KKU-M055</td>
<td>Moderately differentiated</td>
<td>0.001 ± 0.000</td>
<td>0.003 ± 0.002</td>
</tr>
<tr>
<td>KKU-M214</td>
<td>Moderately differentiated</td>
<td>0.003 ± 0.000</td>
<td>0.006 ± 0.003</td>
</tr>
<tr>
<td>KKU-M139</td>
<td>Squamous</td>
<td>0.037 ± 0.009</td>
<td>0.025 ± 0.003</td>
</tr>
<tr>
<td>KKU-100</td>
<td>Poorly differentiated</td>
<td>0.074 ± 0.07</td>
<td>0.10 ± 0.07</td>
</tr>
<tr>
<td>KKU-M213</td>
<td>Adenosquamous</td>
<td>0.12 ± 0.05</td>
<td>0.234 ± 0.225</td>
</tr>
<tr>
<td>KKU-OCA17</td>
<td>Well differentiated</td>
<td>0.57 ± 0.14</td>
<td>0.64 ± 0.15</td>
</tr>
<tr>
<td>KKU-M156</td>
<td>Moderately differentiate</td>
<td>0.64 ± 0.44</td>
<td>1.4 ± 0.6</td>
</tr>
</tbody>
</table>

Data represents mean ± SD of at least three experiments.

Discussion

The in vitro cytotoxic effect of two new chemotherapeutic drugs including paclitaxel and irinotecan towards the seven human intrahepatic CCA cell lines was demonstrated in the present study. Potent cytotoxic activities of paclitaxel and irinotecan observed in those CCA cell lines suggest that these drugs are useful for treatment of cholangiocarcinoma. Our results clearly demonstrated that the CCA cell lines responded well to paclitaxel, an alkaloid derivative, which acts as a mitotic spindle poison and functions to inhibit cell division at G2/M phase of cell cycle. Lower sensitivity towards irinotecan (a DNA topoisomerase I inhibitor) was observed in these CCA cell lines except for KKU-OCA17. It was also noted that KKU-M156 was the least sensitive cell line towards both chemotherapeutic drugs. It has been previously reported that different degrees of sensitivity towards chemotherapeutic agents among various types of CCA cell line were not associated with the histological type of CCA. The results from the present study also clearly revealed that generic formulations and reference formulations of paclitaxel and irinotecan exhibited similar in vitro efficacy on all seven human intrahepatic CCA cell lines.

In conclusions, these in vitro evidences clearly indicate that CCA cell lines are sensitive to both paclitaxel and irinotecan. In addition, the generic formulations (Intaxel® and Irinotel®) have an efficacy on CCA cell lines as similar to their respective reference formulations, Taxol® and Campto®.

References
