Determination of Growth Inhibitory Effect of Gemcitabine on Human Intrahepatic Cholangiocarcinoma Cell lines and Comparison of its Inhibition Between the Generic and Reference Formulation

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Background and Objective: Gemcitabine is one of the most popular drug-of-choices that is currently used for the treatment of cholangiocarcinoma (CCA). However, the study revealing the inhibitory effect of this agent in the series of CCA cell lines established from Thai patients has not been reported. We aim to determine and compare the growth inhibitory effect of generic gemcitabine formulation with the reference formulation on CCA cell lines.

Methods: Seven CCA cell lines established in Srinagarind Hospital, Khon Kaen University were used. A cell growth inhibition by gemcitabine was determined by sulforhodamine B. The IC₅₀ value was expressed as the concentration of drug that caused a 50% growth inhibition comparing with untreated control. The IC₅₀ values of those two formulations were compared using independent t-test.

Results: Growths of KKU-M055, KKU-OCA17 and KKU-M139 CCA cell lines were highly inhibited by gemcitabine (IC₅₀ = 13.35-16.0 µM) whereas KKU-M214 was...
Introduction

Cholangiocarcinoma (CCA) is an uncommon cancer worldwide, but its incidence rate is highest in Northeastern Thailand. There are evidences from clinical studies demonstrating that response of this cancer to conventional chemotherapy is relatively poor. Preclinical study using the in vitro drug testing on CCA cells should be the urgent task to screen new anticancer agents in order to provide the data for selecting the potential chemotherapeutic drugs on treatment CCA. Our recent in vitro study has shown that cytotoxicities of various anticancer agents including anthracyclines, platinum derivatives, 5-fluorouracil, taxanes, vinca-alkaloids, etoposide, irinotecan and mitomycin C on five human intrahepatic CCA cell lines isolated from Thai patients show different degrees of potency varied from high to low sensitivities among those cell lines. To date, various types of anticancer drugs are commercially attainable as a low-cost generic formulation. Recently, Namwat and coworkers have demonstrated that generic formulation of two anticancer drugs, paclitaxel and irinotecan, has a similar efficacy towards CCA cell lines to the corresponding reference formulations.

Gemcitabine (20,20-difluorodeoxycytidine, dFdC, Gemza) is a nucleoside analogue that shows activity against solid tumors and hematologic malignancies both as a single agent and in combination with other chemotherapeutic agents. In Thai CCA patients, gemcitabine seems to be the most promising new agent with consistent data supporting efficacy and tolerability. Therapy with gemcitabine and cisplatin is a well-tolerated therapeutic option for patients with unresectable and metastatic cholangiocarcinoma by achieving a response rate of 22% plus an additional disease stabilization rate of 26% giving an overall disease control rate of 48%. To enhance the effectiveness of gemcitabine in CCA treatment may need a preclinical study including in vitro sensitivity testing and the mechanism of drug response. In addition, the introduction of gemcitabine in treatment of CCA patients who admitted at Srinagarind Hospital is fiscally desirable due to the high-cost problem. Therefore, this study aims to determine and compare the growth inhibitory effect of gemcitabine between its generic and reference formulations in seven human intrahepatic cholangiocarcinoma cell lines. These data may provide an moderately inhibited by this drug (IC_{50} = 36.7 \mu M). These least inhibited growths were found on KKU-100, KKU-M156 and KKU-M213 (IC_{50} = 406-4629 \mu M). The generic (Gramagen) and the reference product (Gemza) formulations were not significantly different in their inhibitory effects on the all seven CCA cell lines.

Conclusions: Although the inhibitory effect of gemcitabine was varied towards seven CCA cell lines, there was no difference in the IC_{50} values of the generic and reference formulations. Our findings indicate that the in vitro efficacy of these two formulations is similar.

Keywords: growth inhibitory effect, gemcitabine, cholangiocarcinoma, generic formulation
alternative for physicians and pharmacists in choosing the chemotherapeutic drug for their patients, particularly when cost-effectiveness is a matter of concern.

**Materials and Methods**

**Chemicals**

Generic formulation of gemcitabine (Gramagen®) was from Sandoz S.A: Buenos Aires, Argentina (lot no. 00104, mfg date 02/2008, exp. date 02/2010). Reference formulation of gemcitabine (Gemza®) was from Lilly France S.A.S, Fegersheim, France (lot no. A447931A, mfg date 21/01/2008, exp date: 19/01/2011).

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 the Faculty of Medicine, Khon Kaen University, 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.

**Growth inhibition assay**

Sulforhodamine B (SRB) assay was used to determine growth inhibition as described previously. In brief, CCA cell lines (4 x 10⁴ 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 the 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.

**Table 1** Growth inhibitory activities of test formulation and reference formulation on CCA cell lines.

<table>
<thead>
<tr>
<th>CCA Cell line</th>
<th>Histological type</th>
<th>Mean IC₅₀ (µM) of gemcitabine Gramagen®</th>
<th>Gemza®</th>
</tr>
</thead>
<tbody>
<tr>
<td>KKU-M055</td>
<td>Moderately differentiated</td>
<td>13.3±1.00</td>
<td>13.3±1.00</td>
</tr>
<tr>
<td>KKU-M17</td>
<td>Well differentiated</td>
<td>13.3±1.00</td>
<td>13.3±1.00</td>
</tr>
<tr>
<td>KKU-M139</td>
<td>Squamous</td>
<td>16.6±1.00</td>
<td>16.6±1.00</td>
</tr>
<tr>
<td>KKU-M214</td>
<td>Moderately differentiated</td>
<td>36.7±1.45</td>
<td>26.7±3.34</td>
</tr>
<tr>
<td>KKU-100</td>
<td>Poorly differentiated</td>
<td>406±92</td>
<td>482±70</td>
</tr>
<tr>
<td>KKU-M156</td>
<td>Moderately differentiated</td>
<td>599±119</td>
<td>478±363</td>
</tr>
<tr>
<td>KKU-M213</td>
<td>Adenosquamous</td>
<td>4629±655</td>
<td>4808±852</td>
</tr>
</tbody>
</table>

Data represents mean±SD of at least three experiments. Statistical analysis between two formulations is not different (P > 0.05) in all tested cell lines.
Statistical analysis

Tests were performed in 3 independent experiments. Data were expressed as mean±S.D. IC\textsubscript{50} value represented the concentration of drugs that inhibited 50% cell growth. Comparison of IC\textsubscript{50} values of generic formulation with reference formulation was analyzed using independent \( t \)-test.

### Results

The growth inhibitory effect of the generic formulations of gemcitabine and its reference formulation, Gemza® against the seven CCA cell lines presented as IC\textsubscript{50} values were shown in Table 1. Results obtained from seven CCA cell lines clearly revealed that the degree of sensitivity to this drug was different. Among these cell lines, KKU-OCA17, KKU-M055 and KKU-M139 showed high sensitivity to gemcitabine with IC\textsubscript{50} value less than 20 \( \mu \text{M} \) whereas KKU-M214 was moderately sensitive (IC\textsubscript{50} values of 36.72 \( \mu \text{M} \)). The cell lines with the least sensitivity were as KKU-100, KKU-M156 and KKU-M213 showing the IC\textsubscript{50} values of 406.0, 598.6 and 4629 \( \mu \text{M} \), respectively. Histological types of CCA were not related to gemcitabine sensitivity. Comparison between Gramagen® and Gemza® demonstrated that there was no significant difference in the IC\textsubscript{50} values observed between the generic and the reference formulations.

### Discussion

The growth inhibitory effect of gemcitabine in the seven human intrahepatic CCA cell lines established from Thai CCA patients is firstly demonstrated in this study. Our results revealed that CCA cell lines posssed various degrees of drug sensitivity - from high (less than 20 \( \mu \text{M} \)) to extremely low (up to 4000 \( \mu \text{M} \)). These data may imply that tumor cells obtained from different patients are distinctly responsible to this drug depending upon the intratumoral drug metabolism\(^3\). In addition, cell lines such as KKU-M213 and KKU-M156 that have a low degree of sensitivity to gemcitabine in this study are also less sensitive to paclitaxel and irinotecan\(^4\). On the other hands, KKU-M055 cell lines that are highly sensitive to most chemotherapeutic drugs\(^4\) also showed the great inhibitory effect on gemcitabine. In addition, when compared the growth inhibitory effect of gemcitabine between its generic and reference formulations we clearly found that both formulations contain a similar \textit{in vitro} efficacy in all seven CCA cell lines. In conclusion, the growth inhibitory effects of gemcitabine in seven CCA cell lines are different from high to low degrees. No difference in the IC\textsubscript{50} values of the generic and reference formulations suggests that the \textit{in vitro} efficacies of these two formulations are very similar. Further investigation such as the histoculture drug response assay may be essential for evaluating the effectiveness of this drug on CCA chemotherapy.

### References