Anti-migrative effect of gambogic acid in human cholangiocarcinoma KKU-M213 cells

ฤทธิ์ยับยั้งการเคลื่อนที่ของสาร gambogic acid ต่อเซลล์มะเร็งท่อน้ำดี KKU-M213 ในคน

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ABSTRACT

Tumor metastasis is the most common cause of death in cancer patients. Cholangiocarcinoma (CCA) is a malignant tumor of bile duct epithelium with a slow growing but rapid and high metastasis. Recently, the anti-invasive effect of gambogic acid (GA) in human breast carcinoma cells was reported. In the present study, we investigated the anti-migrative effect of GA in CCA KKU-M213 cells by wound migration assay. We found that the KKU-M213 cells of the control group migrated into the wound area by 12 hours, whereas in the GA treated cells showed a delay in cell moving into the wound area in a dose-dependent manner. At the 0.6 and 1.2 μM of GA treatments, the migration area of treated cells was significantly decreased compared to the control cells. These results indicated that GA had a potential anti-migrative effect on KKU-M213 cells *in vitro*. Therefore, GA may deserve further exploration as an anti-metastatic agent against CCA.

บทคัดย่อ

การแพร่กระจายของมะเร็งเป็นสาเหตุของการเสียชีวิตที่พบบ่อยที่สุดในผู้ป่วยมะเร็ง มะเร็งท่อน้ำดีเป็นมะเร็งของเซลล์เยื่อบุทางเดินน้ำดี ซึ่งมีการเจริญเติบโตช้าแต่มีความสามารถในการแพร่กระจายสูง เมื่อเร็วๆนี้มีรายงานฤทธิ์ของ gambogic acid (GA) ในการยับยั้งการบุกรุกของเซลล์มะเร็งเต้านมของคน ในการศึกษาครั้งนี้ เราทำการทดสอบฤทธิ์ของ GA ต่อการเคลื่อนที่ของเซลล์มะเร็งท่อน้ำดี KKU-M213 โดยใช้ wound migration assay เราพบว่าเซลล์ KKU-M213 ในกลุ่มควบคุมสามารถเคลื่อนที่ไปยัง wound area ภายใน 12 ชั่วโมง ในขณะที่เซลล์ที่ถูก treated ด้วย GA พบการเคลื่อนที่ของเซลล์ไปยัง wound area ช้าลงแบบ dose-dependent manner เมื่อทำการ treat เซลล์ด้วย GA ที่ความเข้มข้น 0.6 และ 1.2 μM พบว่า migration area ลดลงอย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับเซลล์ในกลุ่มควบคุม จากผลการทดลองแสดงให้เห็นว่า GA มีฤทธิ์ยับยั้งการเคลื่อนที่ของเซลล์ KKU-M213 ในหลอดทดลอง ดังนั้น GA สมควรได้รับการศึกษาเพิ่มเติมเกี่ยวกับฤทธิ์ในการยับยั้งการแพร่กระจายของมะเร็งท่อน้ำดี

Key Words : Gambogic acid, Human cholangiocarcinoma KKU-M213 cells, Cell migration
คำสำคัญ : Gambogic acid เซลล์มะเร็งท่อน้ำดี KKU-M213 และการเคลื่อนที่ของเซลล์

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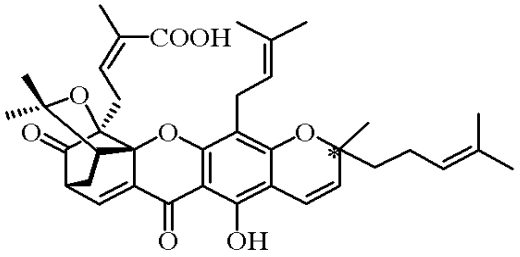
Introduction

Tumor metastasis, the most common cause of death in cancer patients, occurs by a series of events including cell adhesion, migration, invasion, proliferation and vessel formation (Yilmaz et al., 20071). Cholangiocarcinoma (CCA) is a tumor of the bile duct epithelium with a slow growing but rapid and high metastasis (2). The prognosis of CCA patients is extremely poor due to an inability to detect early tumor and the lack of effective chemotherapeutic treatments in the inoperable patients (Hopfner et al., 20083). Therefore, there is a need to develop new effective chemotherapeutic agents for the treatment of advanced and metastatic CCA.

Gambogic acid (GA) (Fig.1), a cage xanthone purified from *Garcinia hanburyi* Hook. f. (family Guttiferae), has been shown to possess an anti-proliferative activity against various cancer cell lines both *in vitro* and *in vivo* (Hahnvajanawong et al., 2010; (Guo et al., 20044). Previous studies demonstrated that the potent anticancer activity of GA is attributed to downregulation of telomerase (Yu et al., 20065), the reduction of CDK7 kinase activity (Yu et al., 20076), inhibition of NF-kB signaling pathway and induction of apoptosis through a transferrin receptor (Kasibhatla et al., 20057). Morover, the anti-invasive effect of GA in human breast cancinoma cells was reported (Qi et al., 20088). Our recent study demonstrated that GA showed selective growth inhibitory effect in CCA KKU-100 and KKU-M156 cell lines by induction of apoptosis through a mitochondria-dependent pathway (Hahnvajanawong et al., 2010). Howover, a potential as an anti-invasive agent of GA in CCA cell lines has not been reported. The present study, we investigated the effect of GA on the migration of the KKU-M213 cell lines.



Gambogic acid

Figure 1 Chemical structure of gambogic acid.

Materials and methods

Chemicals

RPMI 1640 medium, fetal bovine serum (FBS), Penicillin-Streptomycin were purchased from Gibco (Rockville, MD, USA). Gambogic acid was isolated from *Garcinia hanburyi* using bioassay-directed fractionation. All other chemicals were analytical grade.

Human CCA cell line

The human CCA cell line, KKU M213 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, 100U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 incubator.

Cell viability assay

Cell viability was assessed by sulforhodamine B (SRB) assay (Skehan et al., 19909). Briefly, cell (10^4 cells/well) were seeded in a 96 well plate in culture medium with or without 10% FCS and incubated at 37 °C. After 24 hours incubation, cell were treated with DMSO or 0.15-4.8 μM of GA for 24 hours. After removing the medium, cells were fixed with 20% (w/v) TCA at 4 °C for 30 minutes, washed 5 times with distilled water and stained with 0.4% (w/v) SRB in 1% acetic acid at RT for 30 minutes. The

plates were washed 5 times with 1% acetic acid and the bound dye was solubilized with 10 mM Tris buffer, for 5 minutes. The absorbance (OD) was measured by using ELISA plate reader (SUNRISE, TECAN) at 510 nm. The percentage of viable cell was calculated by

$$\% \text{ cell viability} = \frac{\text{OD treated cells (24h)} - \text{OD control cells (0h)}}{\text{OD control cells (24h)} - \text{OD control cells (0h)}} \times 100$$

Wound migration assay

A wound migration assay was performed using a procedure described previously (Jones et al., 199310). Briefly, cells were plated at 70% confluence in growth medium at 37 °C for 24 hours. Confluent monolayers were cultured in conditioned medium for 24 hours and a single scratch wound was created using a micropipette tip, then incubated in conditioned medium in absence or presence of 0.3-1.2 µM GA for 12 hours. After washing with PBS and adding conditioned medium, cell migration into the wound area was monitored by microscopy and digitally photographed. The distance (D) of the wound area were measured on the images and the percentage of migration area was calculated by

$$\% \text{ migrate area} = \frac{\text{D treated cells (0h)} - \text{D treated cells (12h)}}{\text{D control cells (0h)} - \text{D control cells (12h)}} \times 100$$

Statistical analysis

Data were expressed as mean ± standard errors from three independent experiments. Statistical differences were determined by Student's *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.

Results

Effect of GA on the viability of KKU-M213 cells

After treatment of KKU-M213 cells with GA for 24 h, cell viability was assessed. In the presence of serum, GA at different concentrations of GA (0.15, 0.3, 0.6, and 1.2 µM) did not influence the viability of cells (Fig.2A). Moreover, the viability of cells treated with certain concentrations of GA was also not affected in the absence of serum (Fig. 2B). KKU-M213 cells were significantly inhibited at 2.4 µM of GA and completely disappeared at 4.8 µM of GA in both conditions (Fig. 2A, 2B). Therefore, GA at concentrations of 0.3 to 1.2 µM were used for studying a migration activity.

Effect of GA on the migration activity of KKU-M213 cells *in vitro*

Effect of GA on KKU-M213 cell motility was determined by using wound migration assay. Based on the primary experiment, the period of 12h was selected for cell migration (data not shown). In control group KKU-M213 cells migrated into the wound area by 12 h, whereas the GA treated cells showed a delay in cell migrating into the wound area in a dose-dependent manner (Fig. 2C). The migration area of the GA treated cells (at 0.6 and 1.2 µM of GA) was significantly decreased compared to the control cells (Fig.2D)

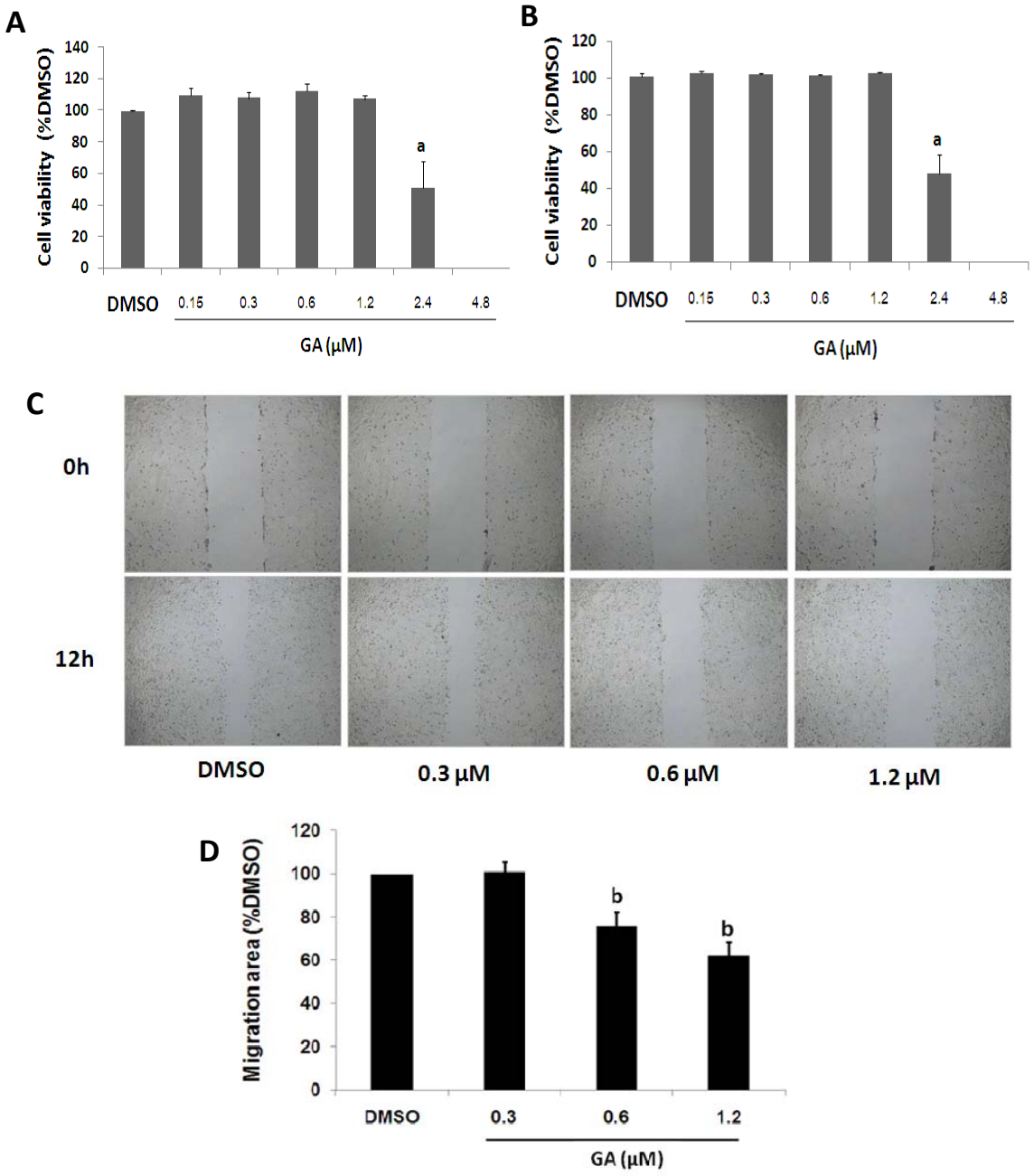


Figure2. (A) Effect of GA on the viability of KKU-M213 cells in the presence of serum. (B) Effect of GA on the viability of KKU-M213 cells in the absence of serum. (C) Photographs of the cell migration of DMSO or GA treated cells. (D) The migration area of DMSO or GA treated cells. Data are presented as the mean ± SE of three independent experiments. b, $P < 0.05$, vs. the DMSO group.

Discussion

Tumor growth, invasion, and metastasis are multistep and complex processes that include cell division and proliferation, proteolytic digestion of the extracellular matrix, cell migration through basement membranes to reach the circulatory system, and remigration and growth of tumors at the metastatic sites (Qi et al., 20088). In this study, we investigated the anti-migrative effect of GA in KKU-M213 cells. Our results demonstrated that GA effectively inhibited the migration of KKU-M213 cells in a dose-dependent manner. Our results are consistent with the previous study in which GA was found to inhibit breast carcinoma cell adhesion, migration and invasion *in vitro* (Qi et al., 20088). The concentrations of GA which inhibited KKU-M213 cell migration in our study were same with in MDA-MB-231 human breast carcinoma cell lines in the study of Qi et al., 2008. This result may be due to the cell types used in both studies are originated from epithelial cell.

Conclusions

Our results indicate that GA had a potential anti-migrative effect on KKU-M213 cells *in vitro*. The effects of GA on proteolytic digestion of the extracellular matrix cell invasion and their molecular mechanisms are under investigating. Therefore, GA may deserve further exploration as an anti-metastatic agent against cholangiocarcinoma.

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