The Inhibitory Effect of Selenomethionine and Sodium Selenite on a Human Cholangiocyte MMNK1 Cell Line

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ABSTRACT

Cholangiocarcinoma (CCA) is a malignant tumor arisen from bile duct epithelial cells. Liver fluke (*Opisthorchis viverrini*) infection is considered as a main cause of CCA in Northeast Thailand. Low Se intake has been found to be associated with the high risk of several cancers. The chemotherapeutic and chemopreventive potentials of several selenium compounds have been studied and proved in many cancers, which draw our attention on the selenium supplements on CCA. This study aimed to demonstrate the inhibitory effect of 2 selenium compounds, selenomethionine (SeMet) and sodium selenite, on cell viability and migration of a human immortal cholangiocyte MMNK1 cell line using sulforhodamine B assay and wound healing assay, respectively. Our results showed that selenium compounds suppressed the cell viability of MMNK1 but they did not affect cell migration. Our data suggest for the role of selenium compounds in CCA chemoprevention.

Keywords: Selenium, Cholangiocarcinoma, MMNK1 cells

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Introduction

Cholangiocarcinoma (CCA) indicates the malignant tumors from the epithelial cells of the biliary tree. According to the tumors' anatomical localizations, CCAs are classified into two subtypes by the third edition of International Classification of Disease to Oncology (ICD-O-3), intrahepatic CCAs and extrahepatic CCAs (Yao *et al.,* 2014).

Regarding to the statistical data worldwide, CCA is relatively rare, but high rates have been reported in the South-East Asia, especially in Northeastern Thailand (Isan area). The CCA incidence in this areas is exceedingly high with age-standardized incidence rates of 37.4 per 100,000 in men and 16.3 per 100,000 in women (Sripa 2008). The etiology of CCA appears in Isan has been studied and considered to be linked to infections, especially with the liver flukes *Clonorchis sinensis* (*C. sinensis*) and *Opisthorchis viverrini* (*O. viverrini*) (Sripa *et al.,* 2007). CCA develops under the chronic inflammation which produces massively oxidative stress due to the consequence of liver fluke infection (Kawanishi *et al.,* 2006; Thanan *et al.,* 2015).

Selenium (Se) is classified as one of the essential trace elements for human body, but is fairly toxic. One of the important physical functions of Se is to be incorporated into selenocysteine (Sec) and inserted into polypeptide chains to form selenoproteins which mainly act as enzymes involved in redox biology (Labunskyy *et al.,* 2014). Numerous *in vitro* and *in vivo* trials have revealed the potential use of different nutritional forms, not only organic but also inorganic forms, of Se as chemopreventive and/or chemotherapeutic drugs or supplements for cancer treatments (Li *et al.,* 2008). Se which is incorporated into the antioxidant system can help cells to maintain the redox status and prevent cells undergo oxidative-stress related cancer.

Objective of this study

This study aimed to demonstrate the inhibitory effects of 2 selenium compounds, selenomethionine (SeMet) and sodium selenite (Na_2SeO_3), on cell viability and migration in the immortal cholangiocyte cell line (MMNK1) that represents as a precancerous cell in order to evaluate the efficacy of selenium compounds for CCA chemoprevention.

Methodology

Cell culture

The human cholangiocyte MMNK1 cell line (transduced with SV40T and hTERT) (Maruyama *et al.,* 2004) kindly given by Professor Naoya Kobayashi (Okayama University, Japan) was used in this study.

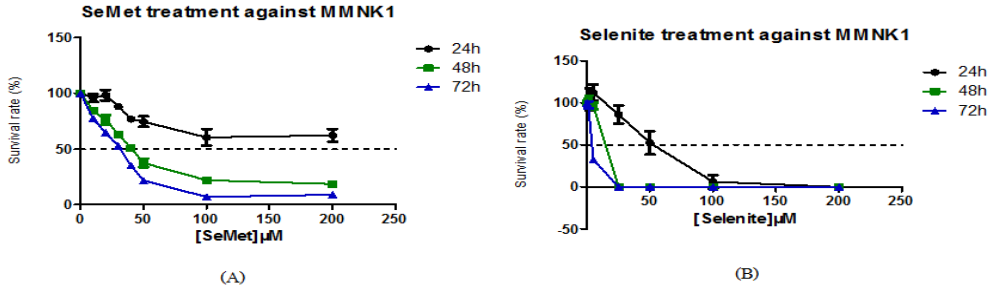

Figure 1 Cytotoxicity of (A) SeMet and (B) Sodium selenite in both dose- and time-dependent manners

time-depending manners.

Cells were cultured in Ham's F-12 medium (Gibco/BRL, Grand Island, NY) supplemented with 44 mM $NaHCO_3$ and 10% fetal bovine serum in a humidified atmosphere containing 5% CO_2. The medium was supplemented with penicillin (100units/ml) and streptomycin (100 mg/ml) (Life Technologies, Inc.).

Wound healing assay

The wound healing assay was used to study directional cell migration *in vitro*. The 2×10^4 CCA cells were seeded in the dish and cultured to complete a confluence. Then a scratch on cell mono layers was made with a pipette tip and the dish was rinsed several times with PBS to remove floating cells before photographed. Cells were continually cultured the under standard conditions for 24 hours. Photos were captured again during cell migration and images were compared in order to quantify the migration rate of the cells. The cell migration was evaluated by counting cells that migrated from the wound edge. The distance of the wound was measured on the images and the migration area was calculated by using the formula: migration area = (area of original wound – area of wound during healing)/area of original wound.

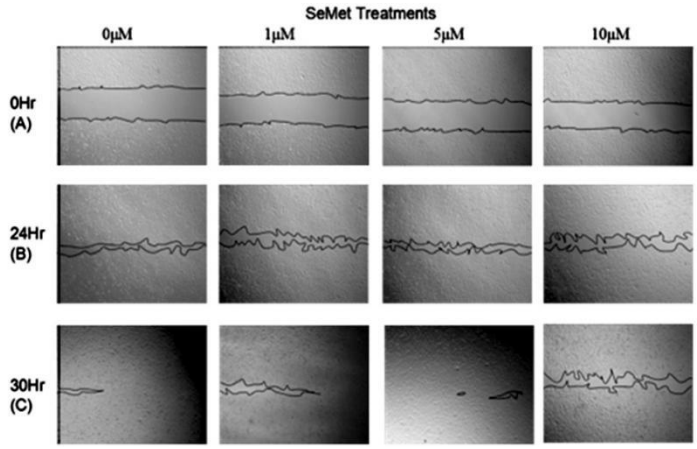

Figure 2 the wound healing ofSeMet-treated MMNK1cell line. The original wound of each condition (A). Wound healing after 24 hours of various doses treatment (B). Wound healing after 30 hours of various doses treatment (C).

Cell viability assay

Briefly, 2×10^3 cells/well were seeded into four 96-well plates and were incubated for 24 h before the treatment with various concentrations of SeMet and sodium selenite solution (prepared with distilled water) for an additional 24, 48 and 72 h. Then, cell mono layers were fixed with 10% (w/v) trichloroacetic acid (TCA) and stained with SRB for 30 min in dark. After, the excess dye was removed by washing repeatedly with 1% (v/v) acetic acid. The protein-bound dye was dissolved in 10 mM Tris base solution for OD detection at 540 nm using the microplate reader.

Statistical analysis

The significance of different data was determined by the Student's *t* test. A P value of less than 0.05 was defined as a statistical significance.

Results

The human cholangiocyte MMNK1 cell line was treated with both SeMet and selenite in both time- and dose-dependent manners. We observed the differential, dose-dependent growth inhibition within 2 compounds. The cytotoxicity of these 2 compounds was shown in Figure 1. The growth of MMNK1 cells could be inhibited on the series of treatment doses from 0 μM to 200 μM. The imaginal lines in the graph indicated the IC_{50} of each compound. The overall mean IC_{50} values of SeMet were over 200 μM (24 h), 50 μM (48 h), and 42 μM (72 h), respectively (Figure 1A). The overall mean IC_{50} values of sodium selenite were 50 μM (24 h), 15 μM (48 h), and 5μM (72 h), respectively (Figure 1B).

SeMet was chosen to perform wound-healing assay. After the wound was made, cells were treated SeMet with various concentrations of 1, 5 and 10 μM and then cultured for 24 h. The result showed that SeMet did not inhibit the MMNK1 cell migration at all time points.

Discussion and Conclusion

Our results indicate that selenium compounds, SeMet and sodium selenite exert an anti-proliferative effect which can inhibit human immortal cholangiocyte growth at the micromolar range. The MMNK1 cell is a precancerous cell that is susceptible to carcinogen or oxidative stress and may be easily transformed to cancer cells (Jusakul *et al.,* 2012). Our data provide that selenium compounds posses the cytotoxic effect on precancerous cells that might prevent CCA development. In addition, SeMet did not affect the cell migration of human cholangiocytes. This effect was opposed to the previous study that showed that SeMet suppressed cancer cell migration (Yoon *et al.,* 2001). In addition, SeMet is less toxic against normal fibroblast cells than MMNK1 cells (unpublished data). This may provide us the clues that it is suitable to be used in CCA chemoprevention. Therefore, our study suggests that selenium compounds that show the inhibitory effect on the growth of immortal cholangiocytes might have an inhibitory effect on CCA cell growth and migration, which are committed to our ongoing investigation.

References

Jusakul A, Loilome W, Namwat N, Haigh WG, Kuver R, Dechakhamphu S, et al. Liver fluke-induced hepatic oxysterols stimulate DNA damage and apoptosis in cultured human cholangiocytes. Mutat Res 2012; 731(1-2):4 8-57.

Kawanishi S, Hiraku Y, Pinlaor S, Ma N. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. Biol Chem 2006; 387(4): 365-72.

Labunskyy VM, Hatfield DL, Gladyshev VN. Selenoproteins: molecular pathways and physiological roles. Physiol Rev 2014; 94(3): 739-77.

Li GX, Lee HJ, Wang Z, Hu H, Liao JD, Watts JC, et al. Superior in vivo inhibitory efficacy of methylseleninic acid against human prostate cancer over selenomethionine or selenite. Carcinogenesis 2008; 29(5): 1005-12.

Maruyama M, Kobayashi N, Westerman KA, Sakaguchi M, Allain JE, Totsugawa T, et al. Establishment of a highly differentiated immortalized human cholangiocyte cell line with SV40T and hTERT. Transplantation 2004; 77(3): 446-51.

Menter DG, Sabichi AL, Lippman SM. Selenium effects on prostate cell growth. Cancer Epidemiol Biomarkers Prev 2000; 9(11): 1171-82.

Sripa B. Concerted action is needed to tackle liver fluke infections in Asia. PLoS Negl Trop Dis 2008; 2(5): e232.

Sripa B, Kaewkes S, Sithithaworn P, Mairiang E, Laha T, Smout M, et al. Liver fluke induces cholangiocarcinoma. PLoS Med 2007; 4(7): e201.

Thanan R, Techasen A, Hou B, Jamnongkan W, Armartmuntree N, Yongvanit P, et al. Development and characterization of a hydrogen peroxide-resistant cholangiocyte cell line: A novel model of oxidative stress-related cholangiocarcinoma genesis. Biochem Biophys Res Commun 2015; 464(1): 182-8.

Yao D, Kunam VK, Li X. A review of the clinical diagnosis and therapy of cholangiocarcinoma. J Int Med Res 2014; 42(1): 3-16.

Yoon SO, Kim MM, Chung AS. Inhibitory effect of selenite on invasion of HT1080 tumor cells. J Biol Chem 2001; 276(23): 20085-92.