Original Article

The Inhibitory Effect of 13-Butoxyberberine Bromide on Migration in Breast Cancer MDA-MB-231 Cells

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Background: 13-butoxyberberine bromide is a berberine derivative that has not been reported on the anti-migration mechanism of breast cancer. Cancer metastasis is the major cause of cancer mortality, about 90% of cancer patients death are caused by cancer metastasis not the primary tumor. Therefore, the compound that could inhibit cancer migration is necessary for development as anti-cancer drug.

Objective: To investigate the effects of 13-butoxyberberine bromide on the anti-migration of breast cancer MDA-MB-231 cells.

Materials and Methods: Cell viability was determined by MTT assay. Wound-healing assay and transwell chamber assay were used to determine cell migration. Protein expression levels were examined by western blot analysis.

Results: 13-Butoxyberberine bromide at sub-toxic concentration (10 µg/mL) inhibited cell migration in MDA-MB-231 treated cells via down-regulation of MMP-2 and MMP-9 expression which play important role in cancer cell metastasis.

Conclusion: 13-Butoxyberberine bromide at 10 µg/mL showed anti-migration effect against MDA-MB-231 cells through inhibition of mediator proteins. These findings could be useful in the development of 13-butoxyberberine bromide to be a novel anti-migration agent for clinical use in the future.

Keywords: Breast cancer, 13-butoxyberberine bromide, Migration, MMPs

J Med Assoc Thai 2019;102(Suppl.6): 12-6
Website: http://www.jmatonline.com

Breast cancer (BC) is the most common cancer in women worldwide and the second leading cause of cancer-related death in developed countries[(1)]. Although, BC usually occurs in developed countries[(2)], however, about half of the BC cases and 60% of the BC deaths occur in developing countries[(3)]. Similarly, Thailand is a developing country, BC is also the most frequently diagnosed cancer in women. Most cancer-related deaths are not due to primary tumor growth but cause by the metastasis of cancer to distant organs[(1)].

Cancer metastasis is the major cause of cancer death, about 90% associate with cancer metastasis not the primary tumor[(4)]. Generally, metastatic processes initiation with basement membrane degradation via secretion of proteolytic enzymes matrix metalloproteinases (MMPs) by cancer cells. Especially, MMP-2 and MMP-9 which play an important role in degradation of extracellular matrix (ECM) proteins in the basement membrane[(5)], finally, induce cancer cell metastasis. The remodeling ECMs by these MMPs support the migration of cancer cells, which facilitates the dissemination of cancer cells to distant organs via blood and lymph circulatory systems. Metastatic cells were established and proliferated to be new secondary tumor[(6)]. Therefore, the compound that could inhibit cancer metastasis is necessary for development as anti-cancer drug.

Berberine is a plant isoquinoline alkaloid with a history of medicinal use in Ayurvedic and Chinese medicine. Berberine can be isolated from many plants including *Hydrastis canadensis* (goldenseal), *Coptis chinensis* (Coptis or goldenthread), *Berberis aquifolium* (Oregon grape), *B. vulgaris* (barberry), and *B. aristata* (tree turmeric)[(7)]. Berberine has shown many pharmacological activities including anti-oxidant, anti-inflammation, anti-bacterial, anti-cholinergic, anti-hypertensive and anti-cancer activities[(8)]. A previous study suggested that berberine has anti-metastatic effect on cancer cells via down regulation of MMP-2 and MMP-9 expression[(9)]. 13-Butoxyberberine bromide, a derivative of bereberine, has been found to have promising anti-breast cancer activity against MCF7 cell line[(10)] but has not been yet studied on the mechanism of its action. Therefore, this study aims to investigate the effects of 13-butoxyberberine bromide on the metastasis in breast cancer MDA-MB-231 cells. This compound is a new derivative that has not been reported to confirm the anti-metastatic effect on cancer cells.

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How to cite this article: Laomethakorn P, Jaitrong M, Samosorn S, Watanapokasin R.The Inhibitory Effect of 13-Butoxyberberine Bromide on Migration in Breast Cancer MDA-MB-231 Cells. J Med Assoc Thai 2019;102(Suppl.6): 12-6.

Materials and Methods

Cell culture

MDA-MB-231 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were maintained in RPMI1640 medium (Invitrogen Life Science, USA) supplemented with 10% fetal bovine serum (GE Healthcare, UK), 100 U/mL penicillin and 100 µg/mL streptomycin (PAA Laboratories, Pasching, Austria). Cells were cultured at 37°C in a humidified atmosphere of 5% CO_2, then, cells were subcultured and the medium was replaced 2 to 3 times/week.

Chemical reagent

13-Butoxyberberine bromide (Berberine derivative) was prepared by Associate Professor Siritron Samosorn (Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand) and dissolved in DMSO before use. 3-(4,5-dimethylthaiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and immobilon western chemiluminescent HRP substrate were purchased from Merck Calbiochem (San Diego, CA, USA). Crystal violet was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell viability assays

Cell viability was determined by MTT assay. Cells were seeded in 96-well plate for 24 h, then treated with 13-butoxyberberine bromide at various concentrations for 24 h. After treatment, the medium was removed and 0.5 mg/mL MTT solution was added to each well and incubated for 2 to 4 h at 37°C. Then, MTT solution was removed and DMSO was added to dissolve the crystals formazan. The absorbance was measured by a microplate reader at 570 nm (Epoch Microplate Spectrophotometer, BioTek Instruments, Inc., USA). The sub-toxic concentration of 13-butoxyberberine bromide that resulted in ≥80% cell survival was chosen for further experiments.

Wound-healing migration assay

The cells were seeded in 6-well plates and allowed to grow until 90% confluence. The wound on the monolayer was created by scratching with plastic tip and washed with PBS to remove cell debris. Then, cells were incubated in fresh medium with or without 10 µg/mL of 13-butoxyberberine bromide. The wounds were photographed at 0, 24 and 48 h, wound distances were measured and calculated by using Image J software (NIH, USA). The percentage of migration was calculated from the area difference in unclosed wound region measured between 0 and 48 h as compared to the untreated control.

Transwell migration assay

The ability of cell migration was measured by transwell chambers assay. The transwell chambers (Merck Millipore Corp.) were inserted into 24-well plate. MDA-MB-231 cells were suspended with serum-free medium with or without 10 µg/mL of 13-butoxyberberine bromide. Then, these cells were seeded in the transwell chambers. The medium contained 10% FBS was added into 24-well plate as a chemoattractant for cell migration induction. After incubation for 24 h, non-migrating cells were removed by using cotton swab and the migrating cells at the lower surface of the membrane were fixed with methanol and stained with 0.5% crystal violet. The migrating cells were photographed under an inverted microscope.

Western blot analysis

Cells were seeded in 6-well plate and then treated with 10 µg/mL of 13-butoxyberberine bromide. Then, cells were lysed with RIPA buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 250 mM NaCl, 0.5% Triton X-100) containing complete mini protease inhibitor cocktail (Roche Diagnostics GmbH, Mannheim, Germany). Protein samples were separated by SDS-PAGE and transferred onto PVDF membrane (Merck Millipore Corp., Merck KGaA). After that the membranes were blocked with 5% non-fat milk for 1 h at room temperature. After blocking, the membranes were incubated overnight at 4°C with primary antibody against MMP-2, MMP-9, and β-Actin. After incubation, the blots were incubated with secondary antibody conjugated with horseradish peroxidase (Cell Signaling Technology, Beverly, MA) for 1 h at room temperature. The protein bands were detected using enhance chemiluminescence (ECL) (Merck Millipore Corp., Merck KGaA) and exposed to CCD camera.

Statistical analysis

All data were obtained from three independent experiments and expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS statistical software (version 17.0). Statistical significance was analyzed by one-way ANOVA.

Results

Effect of 13-butoxyberberine bromide on cell viability in MDA-MB-231 cells

The effect of 13-butoxyberberine bromide on cell viability in MDA-MB-231 cells was determined by MTT assay. The results showed that 13-butoxyberberine bromide decreased cell viability in MDA-MB-231 treated cells at 24 h. This data suggested that 13-butoxyberberine bromide inhibited cell growth in a dose-dependent manner (Figure 1). The sub-toxic concentration of 10 µg/mL of 13-butoxyberberine bromide that resulted in ≥80% cell survival was selected for further experiments.

Effect of 13-butoxyberberine bromide on cell migration inhibition in MDA-MB-231 cells by wound-healing assay

The effect of 13-butoxyberberine bromide on cell migration in MDA-MB-231 cells was examined by wound healing migration assay. The result showed that at 10 µg/mL of 13-butoxyberberine bromide suppressed cell migration in MDA-MB-231 cells compared with non-treated cells

(Figure 2). In addition, 10 µg/mL of 13-butoxyberberine bromide inhibited wound closure by 81.6% compared with control group (0.5% DMSO). These results suggested that 13-butoxyberberine bromide significantly inhibited cell migration of MDA-MB-231 cells.

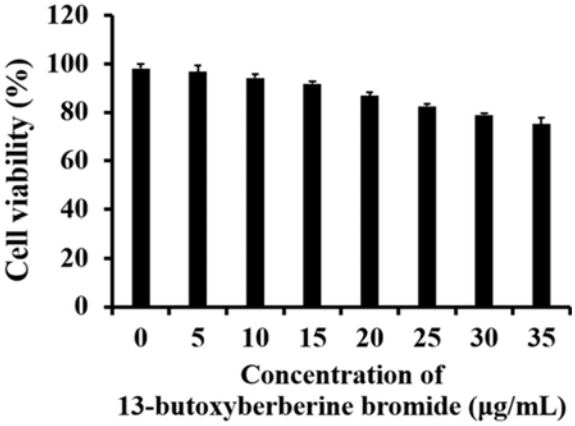

Figure 1. Effect of 13-butoxyberberine bromide on cell viability in MDA-MB-231 cells. Cells were treated with 13-butoxyberberine bromide at different concentrations (0 to 35 µg/mL) for 24 h. The histogram showed percentage of cell viability after treatment with 13-butoxyberberine bromide compared with the control group (0 = 0.5% DMSO). The results represented mean ± SD (n = 3).

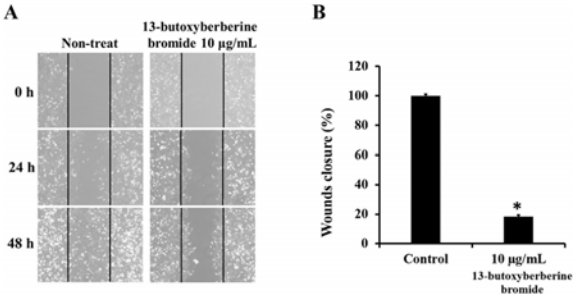

Figure 2. Inhibitory effect of 13-butoxyberberine bromide on wound healing in MDA-MB-231 cells. Cells were scratched and treated with 10 µg/mL 13-butoxyberberine bromide. Then, cells were incubated for 0, 24, and 48 h before observed under inverted microscope and investigated the wound areas. (A) Cell morphology and wound distance were observed under inverted microscope (10X magnification). (B) The histogram demonstrated percentage of wound closure after incubation with 13-butoxyberberine bromide for 48 h. The results represented mean ± SD (n = 3). *assess as significant difference (p<0.05) compared with the control group (0.5% DMSO).

Effect of 13-butoxyberberine bromide on MDA-MB-231 cell migration by transwell chamber assay

The effect of 13-butoxyberberine bromide on cell migration in MDA-MB-231 cells was determined by transwell chamber assay. The results suggested that 10 µg/mL of 13-butoxyberberine bromide significantly reduced cell migration in MDA-MB-231 cells compared with control group (0.5% DMSO) (Figure 3A). Cell migration in MDA-MB-231 cells was suppressed approximately 75.7% after treatment with 10 µg/mL of 13-butoxyberberine bromide (Figure 3B). These results suggested that 13-butoxyberberine bromide significantly inhibited cell migration in MDA-MB-231 cells.

Effect of 13-butoxyberberine bromide on MMP-2 and MMP-9 expression in MDA-MB-231 cells

The effects of 13-butoxyberberine bromide on protein expression levels in MDA-MB-231 cells were determined by western blot analysis. The results showed that 13-butoxyberberine bromide at sub-toxic concentration suppressed MMP-2 and MMP-9 expression compared with control group (0.5% DMSO) (Figure 4). Thus, our study suggested that 13-butoxyberberine bromide showed inhibition of metastasis in MDA-MB-231 cells through MMPs suppression.

Discussion

Metastasis is a complicated mechanism involved in the spread of primary tumor and migration to the target site, which generate secondary tumor at the target organs. The multistep of metastasis cascades begin from the primary tumor develops new blood or lymphatic vessels, these steps are called angiogenesis and lymphangiogenesis. Then the primary tumor escapes from primary site to the target organs. In this step, the cancer cells can invade into blood vessels and migrate to the target organs, which are called cancer cell invasion and migration[11]. Wound healing assay is a simple method which inexpensive to study directional cell migration in vitro. This method starts from wound creation in a cell monolayer to mimic cell migration in vivo. After incubation time, the distance of wound closure is captured and compared with control group. It is suitable for studies on the effects of cell-matrix and cell-cell interactions on cell migration, as well as for whole cell mass migration[12]. In the present study demonstrated that the 13-butoxyberberine bromide showed potential to decrease cancer cells migration in breast cancer MDA-MB-231 cells compared with non-treated cells as showed in Figure 2.

The transwell migration assay is commonly used to study the cell migration ability. During this assay, cells were seeded on the upper layer of a transwell with permeable membrane. And a chemo-attractant solution was placed below the cell permeable membrane to induce cell migration. After

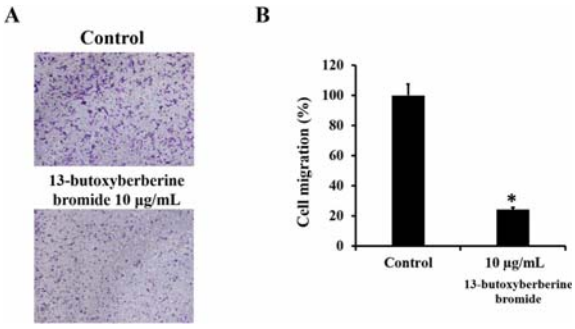

Figure 3. Inhibitory effect of 13-butoxyberberine bromide on cell migration in MDA-MB-231 cells. (A) Morphology of migrated cells after staining with crystal violet under inverted microscope (10X magnification). (B) The histogram showed percentage of migrated cells after incubation with 13-butoxyberberine bromide for 24 h. The results represented mean ± SD (n = 3). *assess as significantly difference (p<0.05) compared with the control group (0.5% DMSO).

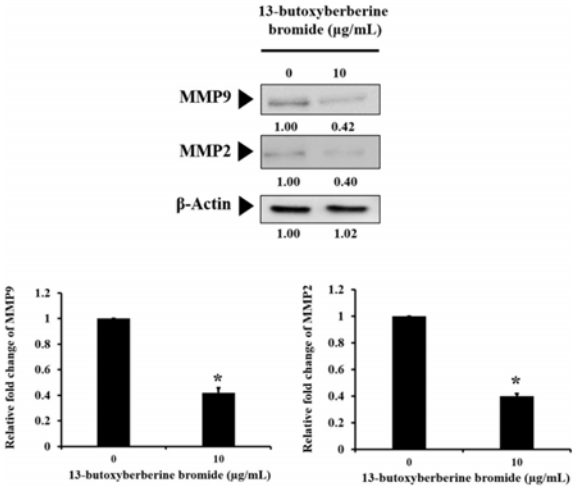

Figure 4. Effect of 13-butoxyberberine bromide on protein expressions in MDA-MB-231 cells. Cells were treated with 10 µg/mL 13-butoxyberberine bromide at 24 h. Protein expression of MMP2, and MMP9 were determined by western blot analysis. The relative band intensities were quantified by Image J analysis densitometer compared with the control group (0.5% DMSO). The results represented mean ± SD (n = 3). *assess as significantly difference (p<0.05) compared with the control group. Actin was used as an internal control.

incubation, cells that have migrated through the membrane were stained and counted[13]. This method is popular to study the ability of cells to sense a particular chemoattractant and migrate through a physical barrier toward it[14]. In the present study, we confirmed the inhibitory effect of 13-butoxyberberine bromide on cancer cell migration by using transwell migration assay. The result indicated that 13-butoxyberberine bromide suppressed MDA-MB-231 cancer cell migration (Figure 3). Moreover, cancer cells invasion and migration are regulated by many molecules of proteins, especially the MMPs proteins. MMPs are group of proteolytic enzyme that play important roles in tumor invasion, metastasis and angiogenesis. Active MMPs are play a critical role in cancer progression to trigger the degradation of ECM and induce tumor invasion and migration[15]. Thus, the increasing of protein expression of MMPs is directly involved in cancer cells invasion, metastasis, and angiogenesis[16]. Many previous studies suggested that berberine compound showed ability to suppress expression of MMPs in mammalian cells such as inflamed skin cells[17] and breast cancer cells[18]. In the present study we investigated protein expression of cell invasion marker proteins in cancer cells by western blot analysis. The authors found that 13-butoxyberberine bromide decreased MMPs expression when compared with the control group (Figure 4). Thus, our study suggested that 13-butoxyberberine bromide inhibited cancer cell metastasis in MDA-MB-231 through decreased the ability of cell migration in wound healing assay and transwell migration assay. Moreover, 13-butoxyberberine bromide induced down regulation of MMP-2 and MMP-9 expression in MDA-MB-231 treated cells.

A previous study suggested that berberine could inhibit cell migration and invasion of highly metastatic prostate cancer cell lines PC-3 and DU145 through suppression of mesenchymal genes expression including *PDGFRB*, *COL1A2*, *BMP7*, *NODAL* and *WNT11* which regulate the development of epithelial-mesenchymal transition (EMT)[19]. In addition, it has been reported that berberine decreased cell migration of breast cancer cell line MCF-7 via down-regulation of *CCR6*, *CCR9*, *CXCR1* and *CXCR4* which are key chemokine receptors[1]. These reports support our findings that 13-butoxyberberine bromide could be a candidate as anti-metastatic agent for breast cancer treatment.

Conclusion

13-butoxyberberine bromide showed the anti-migratory effect in MDA-MB-231 cells. The results indicated that 13-butoxyberberine bromide at 10 µg/mL inhibited cell migration via suppression of MMP-2 and MMP-9 expression. These findings could be useful in the development of 13-butoxyberberine bromide as potent anti-metastatic agent for clinical use in the future.

What is already known on this topic?

Berberine is an effective bioactive compound which has a long history of medicinal use in both Ayurvedic and Chinese medicine. Berberine has shown many

pharmacological activities including anti-oxidant, anti-inflammation, anti-bacterial, anti-cholinergic, anti-diabetic, anti-hypertensive and anti-cancer activities.

What this study adds?

13-butoxyberberine bromide is a new berberine derivative that modified from berberine compound. Berberine derivative, 13-butoxyberberine bromide showed the inhibitory effect on metastasis of breast cancer MDA-MB-231 cells. This compound decreased cell migration in wound healing and transwell assay. Furthermore, 13-butoxyberberine bromide showed cell metastasis inhibition through the downregulation of MMP-2 and MMP-9.

Acknowledgements

This research was supported by The Royal Golden Jubilee PhD Program (grant No. PHD/0028/2556), the Thailand Research Fund. The authors would like to thank the Strategic Wisdom and Research Institute, the Srinakharinwirot University and Research Division, Faculty of Medicine, Srinakharinwirot University, and Faculty of Science Research Unit Grant (No. 405/2560), Srinakharinwirot University and Center of Excellence for Innovation in Chemistry (PERCH-CIC).

Potential conflicts of interest

The authors declare no conflict of interest.

References

1. Ahmadiankia N, Moghaddam HK, Mishan MA, Bahrami AR, Naderi-Meshkin H, Bidkhori HR, et al. Berberine suppresses migration of MCF-7 breast cancer cells through down-regulation of chemokine receptors. Iran J Basic Med Sci 2016;19:125-31.
2. Dai X, Li T, Bai Z, Yang Y, Liu X, Zhan J, et al. Breast cancer intrinsic subtype classification, clinical use and future trends. Am J Cancer Res 2015;5:2929-43.
3. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69-90.
4. Guan X. Cancer metastases: challenges and opportunities. Acta Pharm Sin B 2015;5:402-18.
5. Lynch CC, Matrisian LM. Matrix metalloproteinases in tumor-host cell communication. Differentiation 2002;70:561-73.
6. Liew K, Yong PV, Lim YM, Navaratnam V, Ho AS. 2-Methoxy-1,4-Naphthoquinone (MNQ) suppresses the invasion and migration of a human metastatic breast cancer cell line (MDA-MB-231). Toxicol In Vitro 2014;28:335-9.
7. Kumar A, Ekavali, Chopra K, Mukherjee M, Pottabathini R, Dhull DK. Current knowledge and pharmacological profile of berberine: An update. Eur J Pharmacol 2015;761:288-97.
8. Liu W, Liu P, Tao S, Deng Y, Li X, Lan T, et al. Berberine inhibits aldose reductase and oxidative stress in rat mesangial cells cultured under high glucose. Arch Biochem Biophys 2008;475:128-34.
9. Naveen CR, Gaikwad S, Agrawal-Rajput R. Berberine induces neuronal differentiation through inhibition of cancer stemness and epithelial-mesenchymal transition in neuroblastoma cells. Phytomedicine 2016;23:736-44.
10. Samosorn S, Tanwirat B, Suksamrarn A. Anticancer activity of 13-Alkoxyberberine analogue. Thai Patent Application Number 1101002293, 27 September 2011.
11. Chu SC, Yu CC, Hsu LS, Chen KS, Su MY, Chen PN. Berberine reverses epithelial-to-mesenchymal transition and inhibits metastasis and tumor-induced angiogenesis in human cervical cancer cells. Mol Pharmacol 2014;86:609-23.
12. Rodriguez LG, Wu X, Guan JL. Wound-healing assay. Methods Mol Biol 2005;294:23-9.
13. Senger DR, Perruzzi CA, Streit M, Koteliansky VE, de Fougerolles AR, Detmar M. The alpha (1) beta (1) and alpha (2) beta (1) integrins provide critical support for vascular endothelial growth factor signaling, endothelial cell migration, and tumor angiogenesis. Am J Pathol 2002;160:195-204.
14. Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV. In vitro cell migration and invasion assays. J Vis Exp 2014;(88). doi: 10.3791/51046.
15. Naglich JG, Jure-Kunkel M, Gupta E, Fargnoli J, Henderson AJ, Lewin AC, et al. Inhibition of angiogenesis and metastasis in two murine models by the matrix metalloproteinase inhibitor, BMS-275291. Cancer Res 2001;61:8480-5.
16. Peng PL, Hsieh YS, Wang CJ, Hsu JL, Chou FP. Inhibitory effect of berberine on the invasion of human lung cancer cells via decreased productions of urokinase-plasminogen activator and matrix metalloproteinase-2. Toxicol Appl Pharmacol 2006;214:8-15.
17. Kim S, Kim Y, Kim JE, Cho KH, Chung JH. Berberine inhibits TPA-induced MMP-9 and IL-6 expression in normal human keratinocytes. Phytomedicine 2008;15:340-7.
18. Kim S, Han J, Lee SK, Choi MY, Kim J, Lee J, et al. Berberine suppresses the TPA-induced MMP-1 and MMP-9 expressions through the inhibition of PKC-alpha in breast cancer cells. J Surg Res 2012;176:e21-9.
19. Liu CH, Tang WC, Sia P, Huang CC, Yang PM, Wu MH, et al. Berberine inhibits the metastatic ability of prostate cancer cells by suppressing epithelial-to-mesenchymal transition (EMT)-associated genes with predictive and prognostic relevance. Int J Med Sci 2015;12:63-71.