Anticancer Effects of Terrein in Breast Cancer Cells via Induction of Oxidative Stress

Suntaree Chawsoun BSc*, Faongchat Jarintanan PhD**,
Suchada Jongrungruangchok PhD***, Supinya Pongsunk PhD****, Wanlaya Uthaisang-Tanechpongtamb PhD*

** Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*
*** Faculty of Medical Technology, Rangsit University, Pathumthani, Thailand*
**** Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Rangsit University, Pathumthani, Thailand*
***** Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*

Background: *New anticancer drugs that eradicate specifically to cancer cells are needed to develop. In this study, terrein a compound isolated from Aspergillus terreus, was selected to test for the anticancer activity and oxidative-induced cancer cell death as target mechanism.*
Objective: *To determine the cytotoxicity of terrein comparing between breast cancer and non-cancerous cells and measuring the level of reactive oxygen species (ROS) and glutathione (GSH).*
Material and Method: *MTT assay was used to test the cytotoxic level of terrein in MDA-MB-231 (a triple negative breast cancer cell line) comparing to Vero, a non-cancerous cells. For the amount of ROS, cell permeable fluorescent probe DCFDA was used and detected with flow cytometer, while the level of GSH was determined by using spectrophotometer.*
Results: *The result showed that terrein was cytotoxic to MDA-MB-231 at higher level than in Vero cells, the IC_{50} was at 0.09 and 0.57 mM, respectively. The amount of ROS was increased in a dose-dependent manner, while GSH content was reduced with dose- and time-dependent manner.*
Conclusion: *The possible mechanism to induce breast cancer cell death by terrein is via the induction of oxidative stress and reduction of antioxidant GSH level. Hence, these data support the notion that terrein is an interesting compound to develop as anticancer agent.*

Keywords: *Terrein, Oxidative stress, ROS, Glutathione, Breast cancer*

J Med Assoc Thai 2017; 100 (Suppl. 8): S152-S158
Full text. e-Journal: *http://www.jmatonline.com*

Breast cancer is presently considered as one major public health problem for all nations. For Thailand, the age-standardized incidence rate (ASR) of all cancer in female during 2010 to 2012 reported that breast cancer was found as the most common at 28 per 100,000 females, while cervical and liver cancer found in 14.4 and 12.9 per 100,000 females, respectively. When compared to other Southeast Asian countries, the estimated breast cancer incidence and mortality cases of Thailand ranks third after Indonesia and Philippines in 2012[1]. The high incidence and mortality rates most likely indicates that the patients were detected in the late stage for which treatment was not successful. In addition, the problem of chemotherapeutic drugs resistance and recurrent disease are major obstacles. Therefore, new therapeutic ways or new anticancer agents may be a possible strategy to make treatment more efficient.

Several sources from plants, marine organisms and microorganisms are used to produce anticancer agents. In microorganisms, it has been known that both the bacterial and fungal metabolites are major sources of bioactive compounds. However, there is still not many of the anticancer drugs produced from fungal metabolite. Therefore, this study was aimed to find a new fungal bioactive compound that potentially acts as anticancer agent. With this objective, terrein, a secondary metabolite isolated from *Aspergillus terreus*, has been selected from its variety of biological activities (Fig. 1). The data reported that terrein could act as melanogenesis inhibitors, anti-inflammatory, and anticancer[2-4]. The cytotoxic effect to cancer cells has been shown in prostate, lung, liver, ovarian, head and neck, breast and cervical cancer cells[4-8]. The

Correspondence to:
Uthaisang-Tanechpongtamb W, Department of Biochemistry, Faculty of Medicine, Srinakharinwirot University, 114 Sukhumvit 23, Wattana, Bangkok 10110, Thailand.
Phone: +66-2-6495000 ext. 4609, Fax: +66-2-6495834
E-mail: wanlaya@g.swu.ac.th

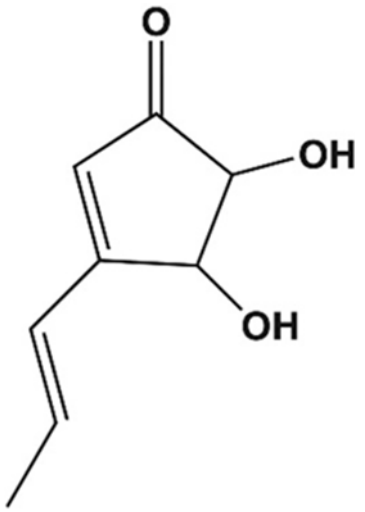

Fig. 1 The structure of terrein.

mechanisms to induce cancer cell death have been proposed but still unclear. As it is already known that the activation of oxidative stress in cancer cells is one promising effect of several anticancer candidates[9,10]. Then, it is possible that oxidative stress may act upstream and cause cancer cell death induced by terrein. Therefore, in this work, we examined the property of terrein as oxidative stress induction by determining the level of reactive oxygen species (ROS) as well as the level of antioxidant glutathione. The model of triple negative breast cancer cells (MDA-MB-231) was selected to use in this study. This subtype is lacking of estrogen receptors, progesterone receptors and human epidermal growth factor receptor 2 (HER2). Then, MDA-MB-231 is particular an aggressive type and more likely to recur than other subtypes of breast cancer[11]. The effect of terrein to this subtype of breast cancer is possibly a new option for breast cancer treatment which would be useful for the development of the anticancer drug in the future.

Material and Method

Materials

DMEM (Dulbecco's Modified Eagle Medium), Fetal Bovine Serum (FBS), 0.25% Trypsin-EDTA and Penicillin-streptomycin was obtained from GIBCO (Invitrogen, USA). 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) was obtained from USB corporation, USA. DCFDA-Cellular Reactive Oxygen Species Detection Assay Kit (ab113851) was purchased from Abcam, UK. Glutathione Assay Kit (CS0260) was purchased from Sigma-Aldrich, Germany. Dimethyl sulfoxide (DMSO) was obtained from Sigma-Aldrich, Germany. General chemicals were purchased from Sigma-Aldrich, Germany.

Preparation of terrein

Terrein was isolated from the culture broth of fungi *A. terreus* CRI301. To prepare the purified compound, the method was followed as previously described[12]. Briefly, ethyl acetate was used as an extraction solvent in the culture broth. After that, the ethyl acetate (EtOAc) extract was concentrated and then fractionated in the Sephadex LH-20 (2 cm inner diameter and 125 cm long) and using methanol (MeOH) as an eluent. Spectroscopic analysis was finally used to confirm the purity and characteristic of the compound.

Cells culture

Breast cancer cells MDA-MB-231 and Vero cells were obtained from ATCC (ATCC, USA). Both cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) and 100 U/ml of penicillin-streptomycin and maintained at 37°C in an atmosphere of humidified air with 5% CO_2.

Cell proliferation assay

The cytotoxicity was tested by seeding MDA-MB-231 ($1.5x10^4$ cells/well) or Vero cells ($1.0x10^4$ cells/well) with medium containing 10% FBS. Then, cells were treated with terrein in DMSO at concentration of 0, 0.075, 0.15, 0.3, 0.625, 1.25 and 2.5 mM, and 0.5% DMSO was used as a vehicle control. After that, cells were incubated for 24 h at 37°C in 5% CO_2. Then, 5 mg/ml of MTT solution was added into each well and incubated further for 3 h. The formazan crystal products were dissolved by addition of 100 µl of DMSO. After 15 min, the amount of colored formazan was determined by measuring optical density (OD) at 595 nm with microplate reader (BioTek Synergy HT, USA). The experiment was performed in triplicates and the percentage of viability was calculated as: % Viability = [OD of treated cells/OD of control cells] x100. The IC_{50} was calculated using program GraphPad Prism 5.

Determination of intracellular reactive oxygen species

The level of reactive oxygen species (ROS) was determined by using DCFDA Cellular Reactive Oxygen Species Detection Assay Kit (Abcam, UK). In this assay, 2', 7'-dichlorofluorescin diacetate (DCFDA)

a cell permeant reagent was used to evaluate the hydroxyl, peroxyl and other ROS activity within the cell. After DCFDA diffusion into the cell, DCFDA is deacetylated by cellular esterases to a non-fluorescent compound which could be later oxidized by ROS and changes into a fluorescence dye 2', 7'-dichlorofluorescein (DCF). The fluorescence generated is proportional to the amount of ROS; hence the increasing level of DCF implies the increasing amount of ROS. To perform the experiment, MDA-MB-231 was seeded at density $2.5x10^4$ cells/well in a 12 well-plate for 24 h. The cells were washed once with phosphate buffer saline (PBS) buffer and stained with 25 µM DCFDA in PBS buffer for 45 min at 37°C. After that, treated samples were added with 100 µl/well of terrein solution at final concentration of 0, 0.05, 0.1, 0.2 and 0.4 mM (which are approximately 0.5X, 1X, 2X and 4X of IC_{50}). For positive control, cells were treated with Tert-butyl Hydrogen Peroxide (TBHP) (Abcam, UK) concentration of 50 mM and incubated for 6 h. All treated samples together with untreated control cells or diluent treated stained cells as well as blank media or buffer only were measured with flow cytometer (Guava easyCyte HT, MERCK, Germany). The increasing amount of DCF fluorescence represented the increasing of ROS.

Determination of glutathione content

The glutathione content assay used Glutathione Assay Kit (Sigma-Aldrich, Germany) that involves oxidation of glutathione (GSH) by the sulfhydryl reagent 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5'-thio-2-nitrobenzoic acid (TNB) which is measured spectrophotometrically at 412 nm. To perform the experiment, MDA-MB-231 cells were seeded into a 6 well-plate at density of $1x10^6$ cells/well and incubated overnight. After that, sample cells were treated with various concentrations of terrein at 0, 0.05, 0.1, 0.2 and 0.4 mM for 6, 12 and 24 h. Then, washed with PBS and suspended the cells in 1 ml of PBS before transferring the cells to a microcentrifuge tube. The samples were centrifuged at 600xg and the supernatants were removed. The volume of the pellet was measured by added 3 volumes of 5% Sulfosalicylic acid solution (5% SSA) (Sigma-Aldrich, Germany) (about 10 µl) to the packed cell pellet and vortex. Then, 150 µl of working mixture solution was added to each well, mixed and incubated for 5 min at room temperature. After that, 50 µl of the diluted NADPH solution were added each well and measured at OD_{412} in the microplate reader (BioTek Synergy HT, USA). The standard curve of reduced glutathione was used to calculate the amount of GSH in each sample.

Statistical analysis

The data were expressed as mean±standard deviation (SD). Statistical significance between tested sample and control group was determined by using one way ANOVA analysis of variance. The *p*-values; $*p<0.05$, $**p<0.01$, $***p<0.001$ were considered significantly different from the control group.

Results

Effect of terrein on the viability of breast cancer (MDA-MB-231) and Vero cells

MTT assay was used in this study for measuring the effect of terrein on cell viability. The concentrations of terrein were used in the range of 0-2.5 mM both in MDA-MB-231 and Vero cells. The results are shown in Fig. 2, which the IC_{50} value of MDA-MB-231 and Vero cells were at 0.09 and 0.57 mM, respectively. A difference of terrein sensitivity in both cell lines was clearly seen at 0.3 mM. Less than 40% was found in MDA-MB-231, while almost 80% of Vero cells are still remained. However, high concentration of terrein (1.25 to 2.5 mM) affected both cells in similar ways which less than 10% were viable.

The effect of terrein on the generation of reactive oxygen species (ROS)

To determine whether terrein could induce a generation of ROS in cancer cells, MDA-MB-231 cells were treated with various concentrations of terrein that

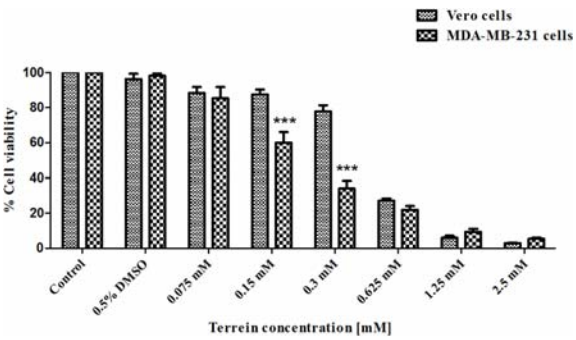

Fig. 2 The effect of terrein on the viability of MDA-MB-231 and Vero cells. Both cell lines were treated with terrein at 0, 0.075, 0.15, 0.3, 0.625, 1.25 and 2.5 mM for 24 h. After that, samples were measured the viability by using MTT assay. $***p<0.001$, versus control.

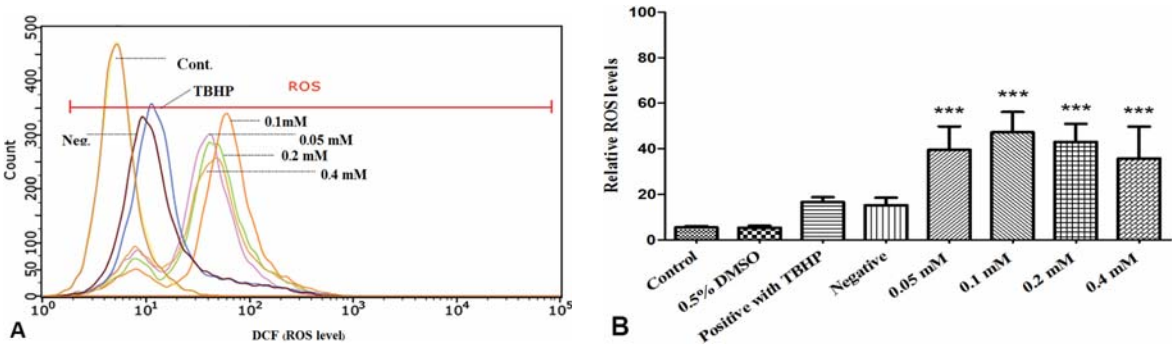

Fig. 3 The effect of terrein on intracellular reactive oxygen species in MDA-MB-231 cells. Cells were treated with terrein at 0, 0.05, 0.1, 0.2 and 0.4 mM for 6 h. The level of ROS was monitored using fluorescent probe (DCFDA) and measured by flow cytometer. (A) a representative of flow cytometry histogram (B) the level of fluorescence intensity of untreated and treated samples. Data presented as means ± SD from three independent experiments, ***p<0.001 versus control.

covered the IC_{50} value (0, 0.05, 0.1, 0.2 and 0.4 mM). The amount of intracellular ROS was monitored by using the fluorescence probe DCFDA and analyzed by flow cytometer. As shown in Fig. 3, data demonstrated that the amount of fluorescence intensity of DCF in the x-axis (represented the level of ROS) were significantly increased in treated samples comparing to untreated control cells.

The effect of terrein on the level of glutathione (GSH)

In order to evaluate the effect of terrein on the level of antioxidant GSH, MDA-MB-231 cells were treated with terrein at 0, 0.05, 0.1, 0.2 and 0.4 mM for 6, 12 and 24 h. As shown in Fig. 4, terrein was affected to the intracellular content of GSH. The level of GSH in the control untreated cells has high level at 6 h and reduced at 12 and 24 h. For the treated samples, the level of GSH (at 6 h) decreased less than 50% after treated with terrein at 0.05 mM, but the level gradually increased when the concentration of terrein was raised from 0.1 to 0.2 mM. At 12 and 24 h, the GSH content seems to decline in a dose-dependent manner. However, at 24 h the GSH content was significantly increased at 0.05 mM comparing to the untreated control cells.

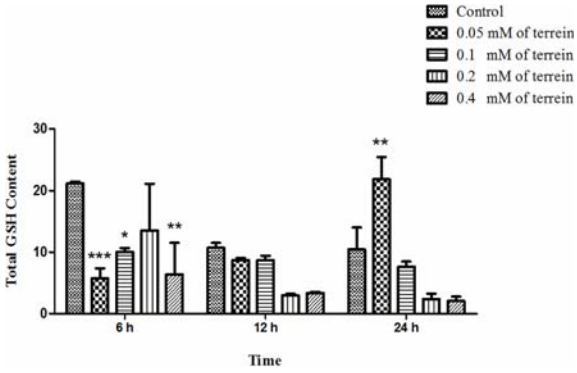

Fig. 4 The effect of terrein on glutathione (GSH) content in MDA-MB-231 cells. Cells were treated with terrein at 0, 0.05, 0.1, 0.2 and 0.4 mM for 6, 12 and 24 h. The amount of GSH content was evaluated by adding diluted NADPH solution and measured at OD_{412} in the microplate reader, *p<0.05, **p<0.01, ***p<0.001, versus control.

Discussion

Natural compounds have been known as a major source of powerful anticancer agents that serve us to combat with cancer. The main sources are mostly from plants, microbes and marine organisms. A number of compounds that are successfully used as chemotherapeutic agents are largely from bacterium such as daunorubicin, doxorubicin, actinomycin D, bleomycin, mitomycin C etc[13]. For fungal metabolites, many of them are ongoing of development in clinical trials. For example, fumagillin produced from *A. fumigates*, function as anti-angiogenesis agent[14]. Phenylahostin, extracted from *A. ustus*, was found to be cytotoxic against several cancer cell lines including lung, cervical, ovary, leukemia, breast and colon[15]. Anguidine, a known mycotoxin belonging to the group of trichothecenes produced from several *Fusarium* species has been shown to inhibit proliferation of leukemia cells[16]. So, from this data, it is showing that microbes are excellent resources of bioactive agents especially to produce the anticancer drug. As shown in this current work, the fungal metabolite, terrein, are

clearly demonstrated the anticancer property. Terrein ($C_8H_{10}O_3$) is a compound isolated from *A. terreus*[17]. The structure contains free hydroxyl groups at positions 4 and 5 of the cyclopentenone ring (Fig. 1). The previous work reported that terrein has several biological activities including anti-inflammatory, melanogenesis inhibitor, antioxidant, and anticancer[2-8]. The cytotoxicity to cancer cells have been demonstrated in lung, breast, leukemia, cervical, head and neck and ovary cancer[4-8]. As clearly seen in this work, terrein exhibited the cytotoxicity to breast cancer cells with IC_{50} at 0.09 mM (Fig. 2). Interestingly, the effect was shown in the breast cancer cells model, MDA-MB-231 which is an aggressive triple negative breast cancer type. This model does not express estrogen receptors (ER), progesterone receptors (PR) and HER2, that cause MDA-MB-231 not well response to hormonal therapy and several chemotherapeutic drugs. Then, the effect of terrein to inhibit this type of breast cancer cells would be a new option of treatment. From MTT assay, the cytotoxic of terrein in non-cancerous Vero cells also evaluated with IC_{50} valued at 0.57 mM (Fig. 2). This cytotoxic effect is estimated about six times less than in breast cancer cells, so terrein could be a good candidate to develop as anticancer agent.

To determine the mechanism used by terrein, oxidative stress induction was selected as a target. It is already known that production of intracellular ROS is an important factor to induce cell death. Also, it has been shown that oxidative stress induction is one promising effect of many anticancer agents such as doxorubicin, paclitaxel, curcumin etc[18-20]. So, it is postulated that oxidative stress may be the mechanism used by terrein to induce cancer cell death. The amount of ROS was measured in treated samples compared to the untreated control cells. As shown in Fig. 3, increasing amount of ROS was found in treated samples which higher than both of untreated control and negative cells (DCFDA staining of untreated cells). Then, this data demonstrated that terrein induces breast cancer cell toxicity via an oxidative stress induction. However, as shown in the previous work that terrein could attenuate the level of ROS in aged human diploid fibroblast (HDF) cells and induce the anti-oxidant molecules such as superoxide dismutase and heme oxygenase-1[7]. Therefore, terrein may acts as both oxidative stress inducer and anti-oxidant activity. This is probably due to a difference of cell type and also the concentration and timing of drug treatment. To further confirm the effect of oxidative stress induced by terrein in breast cancer cells, the content of glutathione was evaluated. GSH is a tripeptide of glutamate, cysteine and glycine that found at high concentration in all mammalian tissues. As shown in Fig. 4, the amount of GSH was found at high concentration in the untreated breast cancer cells at 6 h and declined at 12 and 24 h. The high level of GSH in the untreated control cells at 6 h indicated the essential of GSH for cancer cell survival as shown in several types of cancer cells[21]. The amount of GSH in control cells reduced later at 12 and 24 h, this is probably due to the cancer cells model naturally went through the late apoptotic phase. Since increasing level of GSH is observed to correlate in response to chemotherapeutic agents[22], as shown in the level of GSH in the treated cells at 6 h, the increasing level in a dose-dependent manner was demonstrated. However, the level of GSH exhibited in the opposite way at 12 and 24 h. This is probably due to the effect of terrein that induced ROS production and causing cancer cell death. Several works reported that terrein has ability to trigger cancer cell death via apoptotic mechanism[4,5]. In addition, it is well known that the intrinsic apoptotic pathway is triggered by the ROS, that cause the mitochondrial permeability transition pore and activates the release of cytochrome c into the cytosol[23]. Cellular depletion of GSH is also one importance factor for regulating cell into apoptosis[24]. Therefore, the cells in the later stage are probably enter into apoptotic process and cause changes in the level of GSH.

Conclusion

This work firstly demonstrated that terrein, a fungal metabolite isolated from *A. terreus*, could induce the triple negative breast cancer cells death via oxidative stress induction. The mechanism is clearly seen as terrein could increase the level of ROS and cause a reduction of antioxidant GSH, which potentiate breast cancer cells to enter the death state, especially apoptosis mechanism. The data made us understand more on the function of terrein acting as anticancer agent and would be useful for the development of the anticancer drug for breast cancer treatment in the future.

What is already known about this topic?

It is already known from this topic that terrein, a fungal metabolite extracted from *A. terreus*, has a property of anticancer in several cancer cell types including breast cancer cells. However, the model used in this study and in the previous work is different.

What this study adds?

This work adds more information on the effect of terrein in breast cancer aggressive cell type, MDA-MB-231. Moreover, it is firstly demonstrated that terrein acts as an oxidative stress inducer by increasing the level of ROS and cause a reduction of the antioxidant GSH.

Acknowledgements

This study was supported by Faculty of Medicine (Grant No. 375/2558), Graduate Schools, and Research unit in Biological Activities of Bioactive Compounds, Strategic Wisdom and Research Institute, Srinakharinwirot University.

Potential conflicts of interest

None.

References

1. Youlden DR, Cramb SM, Yip CH, Baade PD. Incidence and mortality of female breast cancer in the Asia-Pacific region. Cancer Biol Med 2014; 11: 101-15.
2. Kim DS, Lee S, Lee HK, Park SH, Ryoo IJ, Yoo ID, et al. The hypopigmentary action of KI-063 (a new tyrosinase inhibitor) combined with terrein. J Pharm Pharmacol 2008; 60: 343-8.
3. Lee JC, Yu MK, Lee R, Lee YH, Jeon JG, Lee MH, et al. Terrein reduces pulpal inflammation in human dental pulp cells. J Endod 2008; 34: 433-7.
4. Liao WY, Shen CN, Lin LH, Yang YL, Han HY, Chen JW, et al. Asperjinone, a nor-neolignan, and terrein, a suppressor of ABCG2-expressing breast cancer cells, from thermophilic Aspergillus terreus. J Nat Prod 2012; 75: 630-5.
5. Porameesanaporn Y, Uthaisang-Tanechpongtamb W, Jarintanan F, Jongrungruangchok S, Thanomsub WB. Terrein induces apoptosis in HeLa human cervical carcinoma cells through p53 and ERK regulation. Oncol Rep 2013; 29: 1600-8.
6. Arakawa M, Someno T, Kawada M, Ikeda D. A new terrein glucoside, a novel inhibitor of angiogenin secretion in tumor angiogenesis. J Antibiot (Tokyo) 2008; 61: 442-8.
7. Lee YH, Lee SJ, Jung JE, Kim JS, Lee NH, Yi HK. Terrein reduces age-related inflammation induced by oxidative stress through Nrf2/ERK1/2/HO-1 signalling in aged HDF cells. Cell Biochem Funct 2015; 33: 479-86.
8. Shibata A, Ibaragi S, Mandai H, Tsumura T, Kishimoto K, Okui T, et al. Synthetic terrein inhibits progression of head and neck cancer by suppressing angiogenin production. Anticancer Res 2016; 36: 2161-8.
9. Tate CR, Rhodes LV, Segar HC, Driver JL, Pounder FN, Burow ME, et al. Targeting triple-negative breast cancer cells with the histone deacetylase inhibitor panobinostat. Breast Cancer Res 2012; 14: R79.
10. Al Oqail MM, Siddiqui MA, Al Sheddi ES, Saquib Q, Musarrat J, Al Khedhairy AA, et al. Verbesina encelioides: cytotoxicity, cell cycle arrest, and oxidative DNA damage in human liver cancer (HepG2) cell line. BMC Complement Altern Med 2016; 16: 126.
11. Bao L, Hazari S, Mehra S, Kaushal D, Moroz K, Dash S. Increased expression of P-glycoprotein and doxorubicin chemoresistance of metastatic breast cancer is regulated by miR-298. Am J Pathol 2012; 180: 2490-503.
12. Demasi M, Felicio AL, Pacheco AO, Leite HG, Lima C, Andrade LH. Studies on terrein as a new class of proteasome inhibitors. J Braz Chem Soc 2010; 21: 299-305.
13. Demain AL, Vaishnav P. Natural products for cancer chemotherapy. Microb Biotechnol 2011; 4: 687-99.
14. Ingber D, Fujita T, Kishimoto S, Sudo K, Kanamaru T, Brem H, et al. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. Nature 1990; 348: 555-7.
15. Kornienko A, Evidente A, Vurro M, Mathieu V, Cimmino A, Evidente M, et al. Toward a cancer drug of fungal origin. Med Res Rev 2015; 35: 937-67.
16. Adler SS, Lowenbraun S, Birch B, Jarrell R, Garrard J. Anguidine: a broad phase II study of the Southeastern Cancer Study Group. Cancer Treat Rep 1984; 68: 423-5.
17. Clutterbuck PW, Raistrick H, Reuter F. Studies in the biochemistry of micro-organisms: The molecular constitution of terrein, a metabolic product of Aspergillus terreus Thom. Biochem J 1937; 31: 987-1002.
18. Marcillat O, Zhang Y, Davies KJ. Oxidative and non-oxidative mechanisms in the inactivation of cardiac mitochondrial electron transport chain components by doxorubicin. Biochem J 1989; 259: 181-9.
19. Wang YF, Chen CY, Chung SF, Chiou YH, Lo HR. Involvement of oxidative stress and caspase activation in paclitaxel-induced apoptosis of primary effusion lymphoma cells. Cancer

Chemother Pharmacol 2004; 54: 322-30.
20. Bhattacharyya S, Mandal D, Sen GS, Pal S, Banerjee S, Lahiry L, et al. Tumor-induced oxidative stress perturbs nuclear factor-kappaB activity-augmenting tumor necrosis factor-alpha-mediated T-cell death: protection by curcumin. Cancer Res 2007; 67: 362-70.
21. Traverso N, Ricciarelli R, Nitti M, Marengo B, Furfaro AL, Pronzato MA, et al. Role of glutathione in cancer progression and chemoresistance. Oxid Med Cell Longev 2013; 2013: 972913.
22. El Sayed SM, Baghdadi H, Zolaly M, Almaramhy HH, Ayat M, Donki JG. The promising anticancer drug 3-bromopyruvate is metabolized through glutathione conjugation which affects chemoresistance and clinical practice: An evidence-based view. Med Hypotheses 2017; 100: 67-77.
23. Gogvadze V, Orrenius S, Zhivotovsky B. Multiple pathways of cytochrome c release from mitochondria in apoptosis. Biochim Biophys Acta 2006; 1757: 639-47.
24. Ortega AL, Mena S, Estrela JM. Glutathione in cancer cell death. Cancers (Basel) 2011; 3: 1285-310.

ฤทธิ์ต้านมะเร็งของสารเทอริอีนในเซลล์มะเร็งเต้านมผ่านการกระตุ้นภาวะเครียดออกซิเดชัน

สุนทรี ชาวสวน, เฟื่องฉัตร จรินทร์ธนันต์, สุชาดา จงรุ่งเรืองโชค, สุภิญญา พงษ์สังข์, วัลยา อุทัยสาง-ธเนศพงศ์ธรรม

ภูมิหลัง: *การพัฒนายาต้านมะเร็งชนิดใหม่ที่มีความจำเพาะต่อเซลล์มะเร็งเป็นสิ่งที่จำเป็นต้องมีการพัฒนาขึ้น ในงานวิจัยนี้ได้ทำการศึกษาการออกฤทธิ์เป็นสารต้านมะเร็งของสารบริสุทธิ์เทอริอีน (Terrein) ที่ได้จากเชื้อรา Aspergillus terreus และเลือกศึกษากลไกการเหนี่ยวนำให้เซลล์มะเร็งตายผ่านการกระตุ้นภาวะเครียดออกซิเดชันเป็นเป้าหมายในงานวิจัย*

วัตถุประสงค์: *ทำการทดสอบความเป็นพิษของสารเทอริอีนต่อเซลล์มะเร็งเต้านมเปรียบเทียบกับตัวแทนเซลล์ปกติและวัดปริมาณของอนุมูลอิสระและสารกลูธาไทโอน*

วัสดุและวิธีการ: *MTT assay ใช้ทดสอบระดับความเป็นพิษของสาร เปรียบเทียบระหว่างเซลล์มะเร็งเต้านม MDA-MB-231 กับตัวแทนของเซลล์ปกติคือ เซลล์ไตลิง Vero และทำการวัดปริมาณสารอนุมูลอิสระโดยย้อมด้วยสีชนิด DCFDA วิเคราะห์ผลด้วยเครื่อง flow cytometer ในขณะที่สารกลูธาไทโอนวัดด้วยเครื่อง spectrophotometer*

ผลการศึกษา: *เทอริอีนมีความเป็นพิษต่อ MDA-MB-231 ที่ IC_{50} เท่ากับ 0.09 มิลลิโมลาร์ และต่อเซลล์ Vero เท่ากับ 0.57 มิลลิโมลาร์ สำหรับผลการเหนี่ยวนำให้เกิดภาวะเครียดออกซิเดชันพบว่าค่าของ ROS ที่เพิ่มขึ้นสัมพันธ์กับความเข้มข้นของสารที่เพิ่มขึ้น ในขณะที่ปริมาณของกลูธาไทโอนมีแนวโน้มลดลงสัมพันธ์กับความเข้มข้นของสาร*

สรุป: *กลไกของสารเทอริอีนในการเหนี่ยวนำให้เซลล์มะเร็งเต้านมตายน่าที่จะผ่านทางการกระตุ้นภาวะเครียด ออกซิเดชัน และการลดลงของสารต้านอนุมูลอิสระกลูธาไทโอน ดังนั้นเทอริอีนจึงเป็นสารที่มีความน่าสนใจในการพัฒนาเป็นยาต้านมะเร็งต่อไปในอนาคต*