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Effect of tanshinone II on cell growth of breast cancer cell line type MCF-7 and MD-MB-231

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Abstract

Breast cancer is the most common form of cancer in women and the leading cause of cancer death in American women, with over 207,090 new cases of invasive breast cancer in women and about 39,840 deaths from breast cancer in 2010. Current therapies for breast cancer usually have variable effectiveness with high toxicity to normal tissues, and breast tumours often develop metastasis and drug resistance. Therefore, searching for effective regimens with minimal side effects remains the top priority in breast cancer research. The objectives of this study were to evaluate the effects of tanshinone II from a Chinese herb, Salvia miltiorrhiza, on the growth of breast cancer cells type MCF-7 and MDA-MB-231.

Key words

Drug design, Salvia miltiorrhiza, apoptosis, diterpenes, natural compounds, Chinese medicine.

Introduction

The extensive chemical diversity of nature provides models and ideas for modern drug design. Naturally occurring compounds consist of structures selected by evolutionary processes to interact with a wide variety of proteins and other biological targets for specific purposes (Yang and Dou, 2010). The use of natural bioactive molecules as modulators of cellular pathways is therapeutically advantageous for several reasons: the selective perturbation of specific features, the temporal control and the reversibility of their action on cell function are all benefits of using natural compounds. On the other hand, the disadvantages may be lack of specificity and cytotoxicity. Natural bioactive molecules play a significant role in cancer since more than 60% of currently available anticancer drugs are natural compounds or are related to them (Gordaliza, 2007). As an example, docetaxel, a second-generation taxane, is one of the most powerful drugs against breast cancer (Saloustros et al., 2008). In developed countries, breast cancer diseases are the second mortality cause after cardiovascular diseases. Current therapies for breast cancer usually have variable effectiveness with high toxicity to normal tissues, and breast tumours often develop metastasis and drug resistance. Therefore, searching for effective regimens with minimal side effects remains the top priority in breast cancer research. Danshen (Salvia miltiorrhiza

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Bunge) has been widely used in traditional Chinese medicine practice for centuries in the treatment of coronary artery disease and cerebrovascular diseases with minimal side effects. In addition to some 20 phenolic acids, 30 diterpene compounds, including the relatively abundant tanshinones (tanshinone I, tanshinone IIA, cryptotanshinone, dihydrotanshinone and tanshinone II), have been isolated from Danshen (Zhou et al., 2005). In addition to their functions in cardiovascular systems, tanshinones have been recently shown to possess some activities against human cancer cells. They are the major diterpenes isolated from Danshen and show cytotoxic effects on cell lines derived from various human carcinomas of the colon, ovary, liver, neuroglia, lung and mouth (Yuan et al,, 2004; Nizamutnova et al., 2008; Yuxian et al., 2009; Lee et al., 2010; Zhang et al., 2010). It is well documented that tanshinone II can induce apoptosis in some human cancer cells, such as leukemia (Sung et al., 1999), human hepatocellular carcinoma (Yuan et al., 2002), and nasopharyngeal carcinoma cells. However, the molecular mechanisms are not yet elucidated. The objectives of this study were to evaluate the activity of tanshinone type II in inhibiting the growth of breast cancer cells type MCF-7 and MDA-MB-231, with the aim of identifying the mechanisms by which tanshinone type II regulates the expression of functional targets.

Materials and methods

Extraction of tanshinone II

The crude tanshinone II used in the present investigation was obtained by extraction with ethanol and n-hexane (1:1, v/v) from S. milthiorriza Bunge. Preparative high speed counter current chromatography (Dynaminc extractions, Slough, UK) with two phase solvent systems composed of n-hexane-ethanol-water (10:7:3, v/v) was successfully performed in a stepwise elution yielding six relatively pure diterpenoids from 300 mg of the crude extract in a single run. The tanshinone II structure was elucidated by 1D and 2D nuclear magnetic resonance spectroscopy (Agilent Technologies, Santa Clara, CA) as well as electrospray ionization mass spectrometry (Agilent Technologies) and comparison with published data.

Cell culture

The human, hormone-independent MDA-MB-231 and the hormone-dependent MCF-7 breast cancer cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA). The cells were placed into 75 cm^3 tissue culture flasks and grown at 37 °C in a humidified atmosphere, in RPMI-1640 medium (Sigma, Milan, Italy), containing 10% heat-inactivated FBS, 1% penicillin-streptomycin (10,000 U/ ml penicillin and 10 mg/ml streptomycin; Sigma). The data presented in this report are from a minimum of three independent experiments.

Cell viability assay

Viability of MDA-MB-231 and MCF-7 cells in response to tanshinone II was tested in dose (0, 1, 10, 100 μmol/l) and time dependent experiments for four days. Cells at

the exponential phase of growth were seeded at 10^4 cells/well in 24 well plates. After different treatments, the amount of viable cells was estimated by MTT assay as follows: 20 μl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) solution were added to each well and incubated for 4 h. The supernatants were aspirated and the formazan crystals in each well were dissolved in DMSO (Sigma) and absorbance at 570 nm was read on a microplate reader (Bio-Rad, Hercules, CA).

Western blot analysis

Breast cancer cells types MDA-Mb-231 and MCF-7 were treated with 10 μM tanshinone II for various periods and lysed in lysis buffer (10 mmol/l Tris, pH 8.0, 120 mmol/l NaCl, 0.5% w/v Nonidet P-40, 1 mmol/l EDTA, and protease inhibitors, *i.e.* 0.1 mmol/l phenylmethylsulfonyl fluoride, 1 mmol/l dithiothreitol, and 1 mg/ml aprotinin) on ice for 30 min. The lysate was centrifuged at 16,873 × *g* (Beckman Coulter Inc.) for 20 min. The supernatant was subjected to 15% SDS-polyacrylamide gel electrophoresis. After transfer of proteins in the gel to a membrane, the membrane was incubated with antibodies for caspase-3, Bax and Bcl2 (1:1,000) and then with horseradish peroxidase-linked secondary antibody and the enhanced chemiluminescence (ECL) detection kit (Amersham, Piscataway, NJ). Protein concentrations were determined with a protein assay kit (Bio-Rad). The amount of target proteins was normalized to the structural protein (tubulin) to control between groups.

Statistical analysis

Values are presented as a mean ± standard deviation (SD). Student's t-test was used to analyze the statistical significance and $p<0.05$ was considered significant for all tests.

Results

As shown in Figs. 1a and 1b it was clearly observed that tanshinone II significantly inhibited the viability of MDA-MB-231 and MCF-7 cell lines. These results were confirmed by morphological analysis of cell growth inhibition observed under a light microscope (Figs. 2a-h). However, with increasing incubation time, a concentration of 100 μmol/l of tanshinone II significantly decreased cell viability. Tanshinone II induced cytotoxicity more effectively in MDA-MB-231 than in MCF-7. To examine if tanshinone II (100 μmol/l) induced cytotoxicity was due to the induction of apoptotic cell death in both cancer cell lines, the levels of the apoptosis- associated proteins, Bcl-2, Bax, and cleaved caspase-3, were detected by western blot analysis. As shown in Figs. 3a,b, tanshinone II (4 days) at a dose of 100 μmol/l increased the levels of caspase 3 cleaved forms in both breast cancer cell lines. Interestingly, and in agreement with the results of MTT test, 100 μmol/l tanshinone II more effectively induced the cleaved form of caspase-3 in MDA-MB-231 than in MCF-7 cells..Moreover, western blot analysis showed that tanshinone II increased pro-apoptotic Bax protein levels and decreased anti-apoptotic Bcl-2 levels in parallel in MCF-7 and MDA-MB-231 cells (Figs. 4a,b).

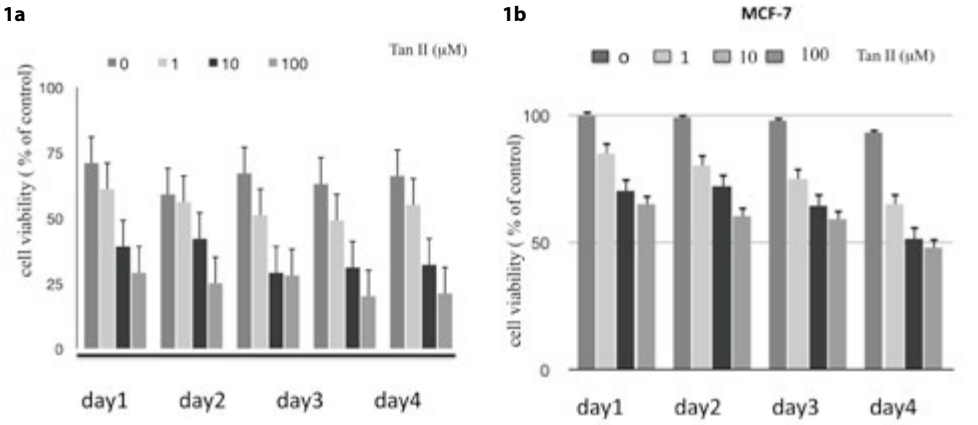

Figure 1a,b – Tanshinone II suppresses cell viabilities of estrogen receptor negative MDA-MB-231 cells (estrogen receptor-negative). The effect of tanshinone II on cell viability was measured in a concentration- and time-dependent manner by MTT. MDA-MB-231 cells were incubated with tanshinone II for 1, 2, 3 and 4 days. It was observed that tanshinone II significantly decreased cell viabilities of cells in a concentration- and time-dependent manner. The data represents the mean ±SD of 3 separate experiments performed in triplicate.

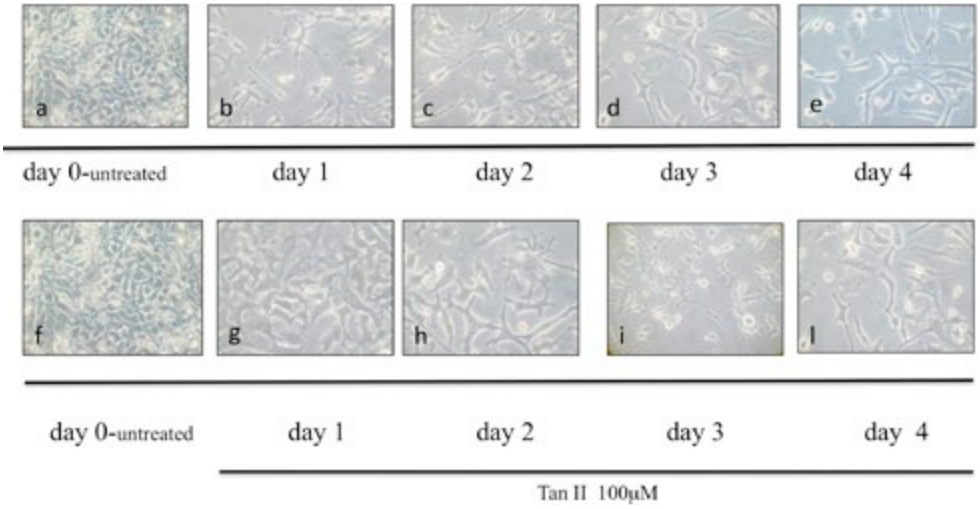

Figure 2 – Morphological analysis by phase contrast light microscopy of the growth inhibition of MDA-MB-231 cells (a-e) and MCF-7 cells (f-l) after treatment with 100 μmol(L tanshinone II .

Discussion

Breast cancer is the most common neoplasm in women in both developed and developing countries. The human breast cancer cell line, MCF-7, is estrogen receptor (ER)-positive, but approximately one-third of breast cancers are ER-negative,

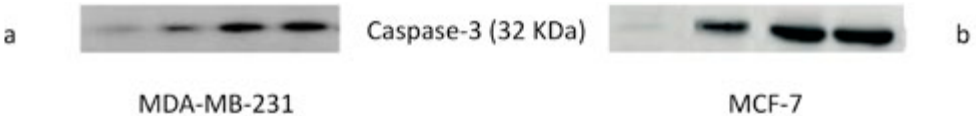

Figure 3 – Tanshinone II activates the caspase-3 pathway in breast cancer cells. Cells were treated with tanshinone II in a concentration-dependent manner for 4 days as described in Materials and methods, and total proteins were extracted.

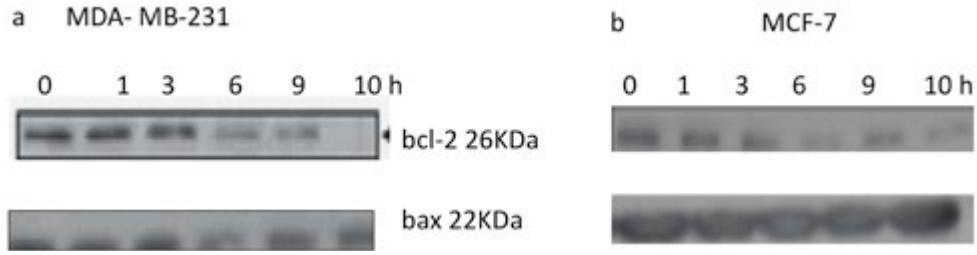

Figure 4 – Tanshinone II activates decreases the Bcl-2/Bax ratio in breast cancer cells in a time-dependent manner.

with the latter carrying a worse prognosis leading to distant metastases. Therefore, it is important to identify new agents that are effective in both ER-positive and -negative breast cancer. Our results demonstrate that tanshinone II not only has a powerful inhibitory effect on the proliferation of ER-positive human breast cancer cells, but also significantly inhibits the growth of human ER-negative cells in vitro. Herbal medications are now used in cancer therapy and several plant-derived compounds are used successfully in clinical practice. The roots of Salvia miltiorrhiza Bunge, generally known as Danshen, have been used in Chinese traditional medicine to treat cardiovascular disorders and hepatitis in Asia for thousands of years. The major bioactive compounds of Danshen, tanshinone I, tanshinone IIA and cryptotanshinone, exhibit diverse biological effects such as antibacterial, antioxidative and anti-inflammatory activity, cytotoxicity and inhibition of platelet aggregation. In this study we found that another bioactive compound of Danshen family such as tanshinone II may exert an inhibitory effect on cell availability in human breast cancer cells by induction of apoptotic cell death. The anti proliferative effect of Tanshinone II probably is mediated by induction of apoptosis process due to the activation of caspase-3 signal. The activation of apoptosis contributes to the suppression of malignant transformation, and has been characterized as a fundamental cellular activity for preventing neoplastic development by eliminating genetically damaged cells, or cells that have been improperly induced to divide by a mitotic stimulus. Thus, induction of apoptosis in cancer cells has become an indicator of cancer treatment response and may hint to reduction of mortality in patients. To reach this goal we have treated two different breast cancer cell lines with Tanshinone II, with the aim to understand if this natural bioactive compound could affect their availability. Our results demonstrate that tan-

shinone II not only has an inhibitory effect on the proliferation of ER-positive (MCF-7) human breast cancer cells, but also significantly inhibits the growth of human ER-negative cells (MDA-MB-231) in vitro. The effect of Tanshinone II is mediated by the induction of apoptosis, which is associated with caspase-3 activation and Bcl2 –Bax proteins, in MCF-7 and MDA- MB-231 cells. Thus, it is reasonable to suggest that the apoptotic potential of tanshinone II is directly related to its ability to alter the ratio of pro-apoptotic to anti-apoptotic proteins in targeted cells. In conclusion, this study determined an anti-cancer effect of tanshinone II mediated by the induction of apoptosis, which is associated with caspase-3 activation and an altered ratio between Bcl-2 and Bax protein levels, in MCF-7 and MDA- MB-231 cells.

References

Gordaliza M. (2007) Natural products as leads to anticancer drugs. Clin. Transl. Oncol. 12: 767-776.

Lee W.Y., Cheung C.C., Liu K.W., Fung K.P., Wong J., Lai P.B., Yeung J.H. (2010) Cytotoxic effects of tanshinones from Salvia miltiorrhiza on doxorubicin-resistant human liver cancer cells. J. Nat. Prod. 5: 854-859.

Nizamutdinova I.T., Lee G.W., Son K.H., Jeon S.J., Kang S.S., Kim Y.S., Lee J.H., Seo H.G., Chang K.C., Kim K.J. (2008) Tanshinone I effectively induces apoptosis in estrogen receptor-positive (MCF-7) and estrogen receptor-negative (MDA-MB-231) breast cancer cells. Int. J. Oncol. 3: 485-491.

Saloustros E., Mavroudis D., Georgoulias V. (2008) Paclitaxel and docetaxel in the treatment of breast cancer. Expert Opin. Pharmacother. 15: 2603-2616.

Sung H.J., Choi S.M., Yoon Y., An K.S. (1999) Tanshinone IIA, an ingredient of Salvia miltiorrhiza BUNGE, induces apoptosis in human leukemia cell lines through the activation of caspase-3. Exp. Mol. Med. 31: 174-178.

Yang H., Dou Q.P. (2010) Targeting apoptosis pathway with natural terpenoids: implications for treatment of breast and prostate cancer. Curr. Drug Targets 11: 733-744.

Yuan S.L., Wei Y.Q., Wang X.J., Xiao F., Li S.F., Zhang J. (2004) Growth inhibition and apoptosis induction of tanshinone II-A on human hepatocellular carcinoma cells. World J. Gastroenterol. 10: 2024-2028.

Yuxian X., Feng T., Ren L., Zhengcai L. (2009) Tanshinone II-A inhibits invasion and metastasis of human hepatocellular carcinoma cells in vitro and in vivo. Tumori 6: 789-795.

Zhang X., Zhang P.R., Chen J., Lü Q. (2010) A study on the effect of Tanshinone II A against human breast cancer in vivo. Sichuan Da Xue Xue Bao Yi Xue Ban 1: 62-67.

Zhou L, Zuo Z, Chow MS. (2005) Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol 12: 1345-59.