

Original Article

Artemether effect and its interaction with vincristine and doxorubicin on human breast carcinoma MCF-7 cells

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Abstract

Introduction: For thousands of years, plants have been used as the main source of drug worldwide. Recently, it has been found out that the plant *Artemisia annua* and especially its derivatives such as artemether have anticancer properties.

Methods: In this study, the anticancer effect of artemether on MCF-7 breast cancer cell line was examined. MTT assay was used to assess the viability of cancer cells.

Results: The data showed that artemether (100-300 nM) resulted in MCF-7 growth inhibition. Artemether plus vincristine or doxorubicin compared to each drug alone showed significant cytotoxic effects. Over 50 percent of cells were killed in combination of non-effective doses of artemether and doxorubicin (50 and 160 nM, respectively).

Conclusion: Taken together, the combined therapy of artemether and anticancer drugs would be a promising strategy for breast cancer but the effectiveness of such combination therapy needs to be verified by experimental and clinical investigations.

Keywords:

Breast cancer cell line;
MCF-7;
Artemether;
Cell viability;
Chemotherapy

Received: 23 Feb 2016

Accepted: 9 Jun 2016

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Introduction

Today, there are four major types of anticancer compounds derived from plants. These types are listed based on their oncological medical properties. These four categories include vinca alkaloids (vinblastine and vincristine), opipodophyladoxinelingan (teniposide and etoposide), taxanditerpenoid (paclitaxel and docetaxel), and derivatives of camptothecinquinoline alkaloids (topotecan and irinotecan) (Russo et al., 2010; Cragg and Newman, 2005; Karuppusamy, 2009; Pan et al., 2010). Recently, it has been shown that artemisinin and its derivatives such as

artemether have anticancer properties (Wu et al., 2009; Jiao et al., 2007; Bhakuni et al., 2001). Artemisinin scientifically known as *Artemesia Annua* is derived from *Artemisia Annua* or Kinghaosu, which is a eudicots type of asteralea growing in Europe and Asia.

In 1972, the active substance of this plant (artemisinin), which is a type of sesquiterpene lactones, was extracted. This substance contains an endoperoxide bridge and is produced semi-synthetically. It is used as an anti-malarian drug in tropical regions (Bhakuni et al., 2001; Gueri et al., 2002). Artemether is methyl ether derived from artemisinin and is used actively to treat blood schizont (Barnes et al., 2009).

In mice, artemether inhibits angiogenesis and migration of cancer cells associated with brain tumor (Wu et al., 2009). It can also inhibit the cell cycle at phase G2/M in ovarian cancer cells (Jiao et al., 2007). Cancer is a complex genetic disease which is the second cause of death in the developed and developing countries after cardiovascular diseases (Siegel et al., 2012). In this study, the anticancer effect of artemether on breast cancer MCF-7 cell lines was examined. Since anticancer drugs have numerous side effects which reduce treatment efficiency, finding a way for increasing the therapeutic effect of anticancer drugs at lower dosages is of great importance. Therefore, the aim of this study was to evaluate the therapeutic effect of combination of anticancer drugs doxorubicin and vincristine with artemether against MCF-7 cancer cell line.

Materials and methods

Materials

Cell culture reagents, penicillin-streptomycin solution, trypsin EDTA, fetal bovine serum (FBS), and heat-inactivated horse serum (HS) were obtained from Biosera Co. (East Sussex, UK). Culture flasks and dishes were acquired from SPL Life sciences Inc. (Gyeonggi-Do, South Korea). 3-[4,5-Dimethyl-2-thiazolyl]-2,5-diphenyl-2-tetrazolium bromide (MTT), doxorubicin and vincristine were purchased from Sigma (St. Louis, MI, USA). Artemether was a kindly gift from Tarbiat Modares University of Tehran.

Cell culture

MCF-7 (human breast adenocarcinoma cell line) cells were obtained from National Cell Bank of Iran (NCBI). The cells were grown with Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL). They were maintained at 37°C in a 5% CO₂ atmosphere. Growth medium was changed three times a week. Cells were plated at the density of 5000 per well in a 96-microplate well for the MTT assays. Then, the cells were incubated with different concentration of the extract alone or in combination with anticancer drugs.

MTT assay

Cellular viability was assessed by the reduction of 2-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

bromide (MTT) to formazan. The MTT assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability (Denizot and Lang, 1986). This method is now widely accepted as a reliable way to examine cell toxicity. MTT was dissolved in PBS and added to the culture at final concentration of 0.5 mg/mL. After additional 2 h incubation at 37°C, the media were carefully removed and 100 µl DMSO was added to each well, and the absorbance (OD) values were determined by spectrophotometry at 490 nm with microplate reader (Eliza MAT 2000, DRG Instruments, GmbH). Each experiment was performed 5-6 independent times. Results were expressed as percentages of control.

Statistical analysis

The results are expressed as mean ± SEM. The differences in mean cell viability assays between experimental groups were determined by one-way ANOVA, followed by Tukey HSD test. P<0.05 was considered significant.

Results

Effect of artemether on MCF-7 proliferation

The data showed that 24 h treatment of artemether at concentration of 100-300 nM induced significant and gradual toxic effects on MCF-7 cells. Artemether had no significant toxic effect on MCF-7 cells viability at doses of 10 and 50 nM. Therefore, such doses were used in the next step of experiments for the evaluation of its synergic effect with vincristine and doxorubicin.

Effects of artemether in combination with vincristine or doxorubicin on MCF-7 cell viability

To investigate the synergic effect of artemether with anticancer drugs, the non-effective doses of artemether (10 and 50 nM) were combined with low efficiency dosage of vincristine (75 and 150 nM) or doxorubicin (80 and 160 nM). The results showed that combination therapy had significant toxic effect as compared to each drug alone. As shown in Figure 2, 10 and 50 nM of artemether significantly potentiated the effect of vincristine. Surprisingly, 50 nM artemether in combination with 75 or 150 nM vincristine had a potent cytotoxic effect which was greater than those observed in cells treated with

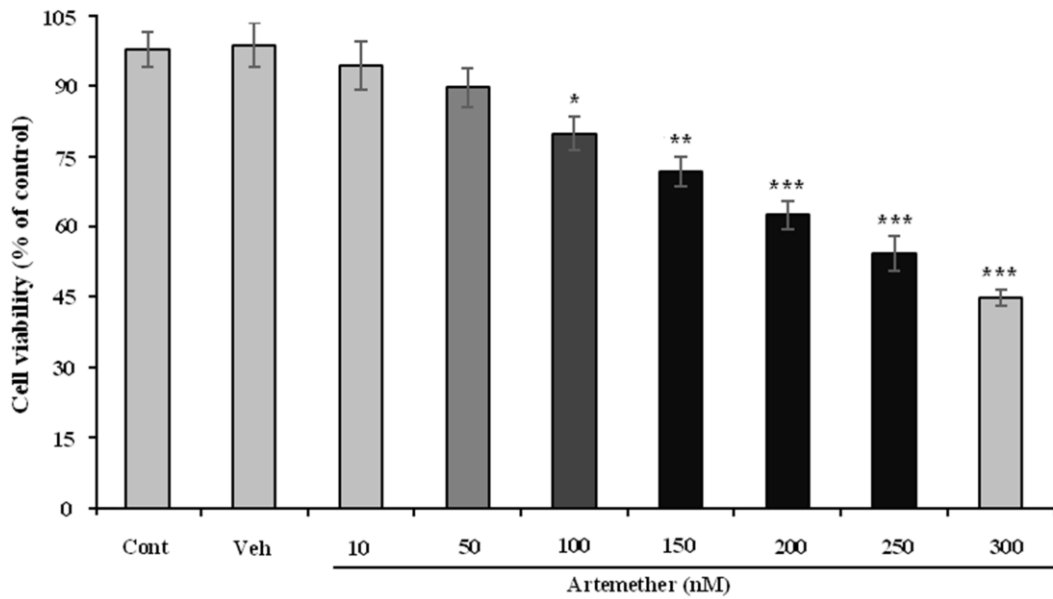


Fig.1. Effects of different concentrations (nM) of artemether on MCF-7 cancer cells viability determined by MTT assay. The extract has a dose dependent toxic effect on cancer cells. Data are expressed as mean \pm SEM; n=6 wells for each group; *P<0.05, **P<0.01 and ***P<0.001 versus control non-treated (Cont) and vehicle-treated (Veh) cells.

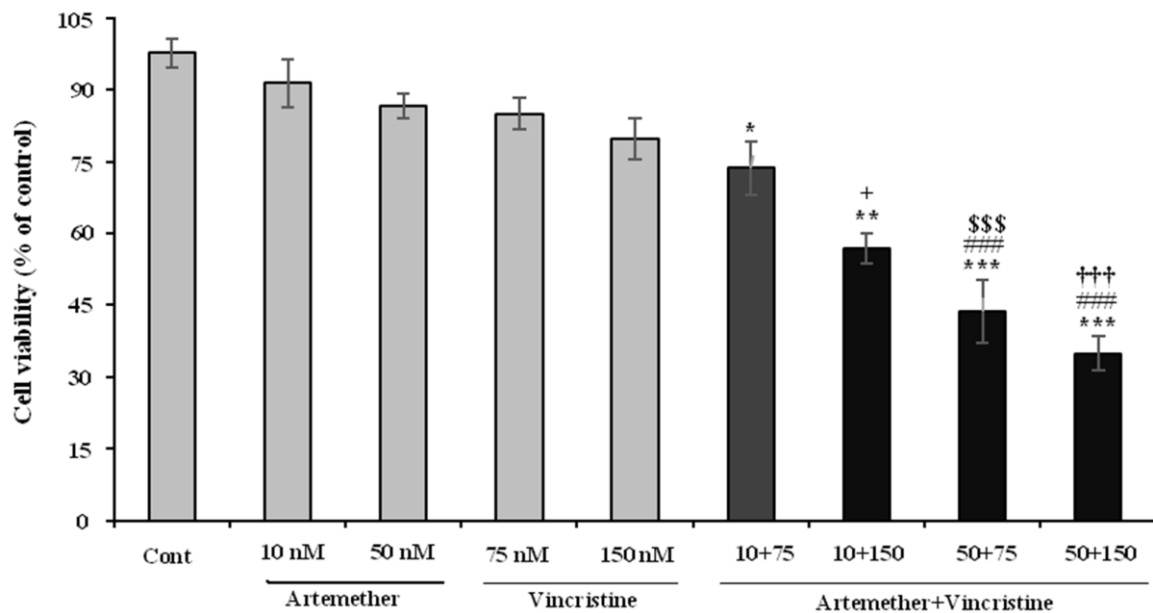


Fig.2. Effect of non-effective (10 and 50 nM) doses of artemether alone or in combination with 75 or 150 nM of vincristine on MCF-7 cell viability determined by MTT assay. Data are expressed as mean \pm SEM; n=6 wells for each group. *P<0.05, **P<0.01 and ***P<0.001 versus control cells. +P<0.05, versus 10 nM artemether-treated group ###P<0.001 versus 50 nM artemether-treated cells. \$\$\$ P<0.001 versus 75 nM vincristine alone. †††P<0.001 versus 150 nM vincristine alone.

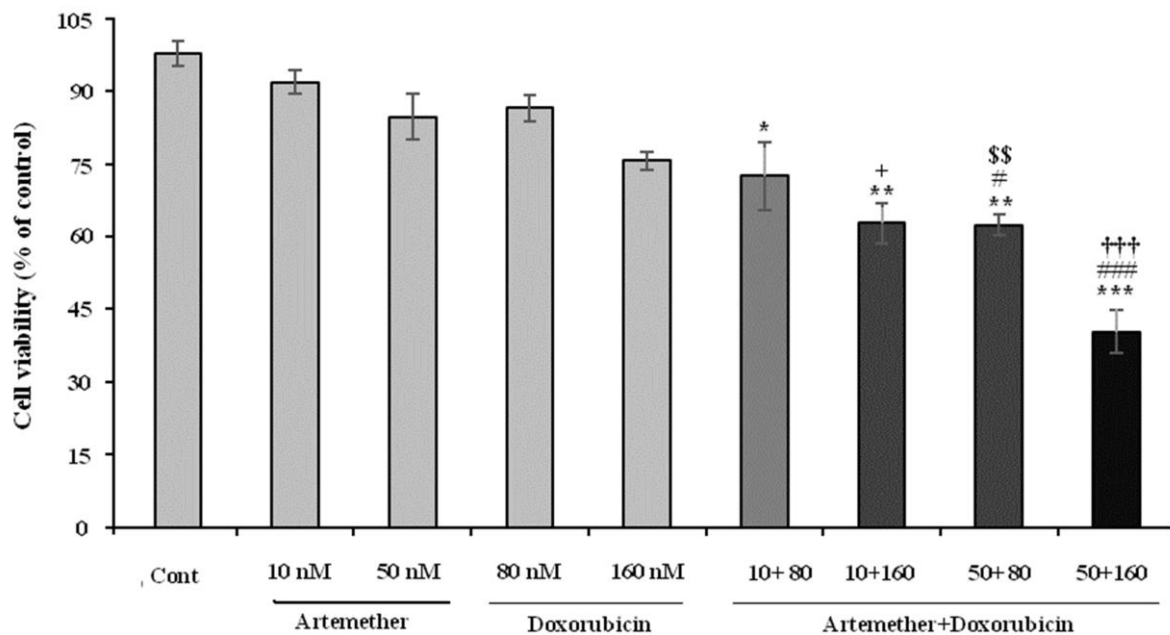


Fig.3. Effect of non-effective (10 and 50 nM) doses of artemether alone or in combination with 80 or 160 nM of doxorubicine on MCF-7 cell viability determined by MTT assay. Data are expressed as mean \pm SEM; n=6 wells for each group. *P<0.05, **P<0.01 and ***P<0.001 versus control group. +P<0.05, versus 10 nM artemether-treated group. ##P<0.01 and ###P<0.001 versus 50 nM artemether-treated cells. \$\$\$ P<0.001 versus 80 nM doxorubicin and ††† P<0.001 versus 160 nM doxorubicin alone.

10 nM artemether plus different doses of vincristine (Figure 2).

To examine the synergic effect of artemether and doxorubicin on the MCF-7 cell viability, the cells were exposed to control medium, non-effective doses of artemether (10 and 50 nM), and doxorubicin alone (80 or 160 nM) or in combination for 24 h. The data showed that artemether and doxorubicinco-treatment markedly killed the cells and decreased cell viability (Figure 3). 50 percent of cells were killed in combination of 50 nM artemether plus 160 nM doxorubicin.

Conclusion

The data from these experiments indicated that artemether has anticancer properties on MCF-7 cancer cell line and possesses a synergic effect with doxorubicin and vincristine.

Despite scientific progress in the understanding of tumorigenesis and molecular methods and techniques to treat cancer, chemotherapy is still considered as one of the most important used

methods in the treatment of cancer (Hanahan and Weinberg, 2011; Stratton, 2011). Vincristine, which is known as leurocristine, is a vinca alkaloid derived from *Vinca*, scientifically known as *Catharanthus roseus*. This drug is a mitosis inhibitor and is widely used in chemotherapy. It prevents polymerization and formation of microtubules. Therefore, vincristine affects cells with rapid mitosis such as cancerous cells. Side effects of this drug include peripheral neuropathy, constipation, and hair loss (Holland et al., 1973; Himes et al., 1976; Kaufman et al., 1976). Doxorubicin is a chemotherapy drug of the anthracycline family. Anthracyclines are antibiotics which are among the most effective and common cytotoxic drugs used for the treatment of tumors. Doxorubicin with the commercial name adriamycin is originally derived from *Streptomyces peucetius* mutans. The main mechanism of action of doxorubicin is intercalation into DNA and disruption of topoisomerase function. It also connects to the iron within cells and leads to the production of free radicals. Doxorubicin-treated patients show complications such as lethargy, hair loss, fatigue,

decreased immunity, and cardiac conditions similar to patients treated with other chemotherapy drugs (Frederick et al., 1990; Dobbs et al., 1995; Barnab  t al., 2002).

Since one of the problems in chemotherapy is side effects, especially toxic effects on normal cells, reducing dose of these medicines with an increased sensitivity of cancer cells to lower dosages will be of great help in mitigating the side effects and improving effectiveness (Surh, 2003; Aggarwal et al., 2004). One of the methods to reduce the therapeutic doses is the combination of different medicines. Some herbal agents have anticancer properties and prevent cancer through several mechanisms. Some of these compounds such as artemether with safety in terms of health conditions and limited side effects provide potential candidates to be combined with anticancer drugs such as vincristine and doxorubicin. These combinations can potentially lower the dose prescribing and minimize the side effects.

Our results showed that artemether possesses anticancer effects on MCF-7 cancer cells. It can also potentiate the toxic effects of vincristine and doxorubicin at their non-effective doses. It is recommended to carry out more studies on the mechanism of this drug and its combination with anticancer agents.

Acknowledgments

This work was supported by funds from Shahid Bahonar University of Kerman.

Conflict of interest

The authors have declared no conflict of interest.

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