Antineoplastic effect of β-elemene on prostate cancer cells and other types of solid tumour cells

Objectives: β-Elemene, a natural compound extracted from over 50 different Chinese medicinal herbs and plants, has been effective in the treatment of hyperplastic and proliferative disorders such as prostatic hypertrophy, hysteromyoma and neoplasms. Our previous studies have demonstrated that β-elemene exhibits strong inhibitory activity in ovarian cancer cells. The aim of the present study was to assess the effect of β-elemene on prostate cancer cells as well as other types of tumour cells and to determine whether the effect of β-elemene on prostate cancer cell death was mediated through the induction of apoptosis.

Methods: The MTT assay was used to evaluate the ability of β-elemene to inhibit cellular proliferation in cancer cells. Cellular apoptosis was assessed by annexin V binding, TUNEL and ELISA-based assays. Caspase activity was measured using a caspases assay kit. The protein levels of Bcl-2, caspases, cytochrome c and poly(ADP-ribose) polymerase (PARP) were analysed by Western blotting.

Key findings: Here, we showed that β-elemene had an antiproliferative effect on androgen-insensitive prostate carcinoma DU145 and PC-3 cells. Treatment with β-elemene also inhibited the growth of brain, breast, cervical, colon and lung carcinoma cells. The effect of β-elemene on cancer cells was dose dependent, with IC50 values ranging from 47 to 95 µg/ml (230–465 µm). TUNEL assay and flow cytometric analysis using annexin V/propidium iodide staining revealed that the percentage of apoptotic prostate cancer cells was increased by β-elemene in a dose- and time-dependent manner. Moreover, β-elemene exposure resulted in a decreased Bcl-2 protein level, increased cytochrome c release, and activated PARP and caspase-3, -7, -9, and -10 in prostate cancer cells.

Conclusions: Overall, these findings suggest that β-elemene exerts broad-spectrum antitumour activity against many types of solid carcinoma and supports a proposal of β-elemene as a new potentially therapeutic drug for castration-resistant prostate cancer and other solid tumours.

Plants
Over 50 plants contain β-elemene [1] and grow in tropical areas around the world and include Rhizoma zeodaria, Radix inulae, Radix ginseng,
Curcuma wenyujin, Cymbopogon citrates, Cymbopogon winterianus Jowitt, Zhangzhou Aglaia odorata flowers and leaves, Fuzhou Aglaia odorata flower, Chuning Aglaia odorata flower and leaves, Yibin geranium leaves, Kunmin geranium leaves, Litchi chenensis cinnamonmi folium, dry Lauris nobilis, Citrus limona leaves, Vitis vinifera grape leaves, Clauensia lansium leaves, Fortunella margarita leaves, Fortunella odoranta, C. Wenyunjin Chen and Magnolia sieboldi among others.[2] In China, β-elemene, the major active component of elemene, has been used effectively in the treatment of hyperplastic and proliferative disorders, including prostatic hypertrophy, hysteromyoma and neoplasms, and one of its preparation formulations (85% β-elemene) has been approved by the State Food and Drug Administration of China for the treatment of primary and secondary brain tumours and other carcinomas.[3] The major advantages of β-elemene as an anticancer drug are that it has antitumour activity toward a broad spectrum of cancers, including leukaemia, it is associated with a low level of toxicity, and it is well tolerated by cancer patients[3-9] Our previous studies have demonstrated that β-elemene exhibits strong inhibitory activity in ovarian cancer cells and non-small cell lung cancer cells.[5-8]

Discussion
Although testosterone depletion remains the gold standard for advanced-stage hormone-sensitive disease, castration-resistant prostate cancer is a conundrum. Thus, the development of promising novel chemotherapeutic agents for castration-recurrent prostate cancer is a high priority. Recently there has been a shifting focus towards finding natural compounds that may prevent and treat prostate cancer.[35] For example, Albrecht and colleagues found that proliferation and invasion of LNCaP, DU145 and PC-3 prostate cancer cells in vitro could be significantly reduced in the presence of polyphenols extracted from various parts of the pomegranate fruit.[10] Malik and coworkers evaluated a crude pomegranate fruit extract (PFE) containing ellagic acid, the main polyphenol in pomegranate, for its anti-proliferative and pro-apoptotic properties and found that it caused both cell growth inhibition and apoptosis in a dose-dependent manner in androgen-insensitive PC-3 cells.[11-12] Oral administration of PFE to mice implanted with androgen-sensitive prostate cancer cells resulted in inhibition of tumour growth, with a significant decrease in serum PSA levels. A number of clinical trials are ongoing, focusing on the potential of other phytochemicals, such as green tea catechins, curcumin, resveratrol and genistein, in the treatment of prostate cancer and other tumours.[13-17]
In the present study, we demonstrate for the first time that β-elemene, a natural compound, inhibits the in-vitro growth and proliferation of the
human androgen-insensitive prostate cancer cell lines DU145 and PC-3 in a dose-dependent manner. β-Elemene treatment of prostate cancer cells resulted in down-regulated Bcl-2 expression, enhanced cytochrome c release, activated caspase-3,-7,-9 and -10, and cleaved PARP, suggesting that β-elemene triggers cell death in prostate cancer cells, at least in part, through an apoptotic pathway mediated by the release of mitochondrial cytochrome c. We also show that β-elemene exhibits strong antitumour efficacy in brain, breast, cervical, colon and lung carcinoma cells. These results are consistent with previous findings that β-elemene is a potent inhibitory agent against several types of solid tumours, but has only moderate effects on normal and non-cancerous cells.[3-9]

β-Elemene (98% preparation formulation) has been shown to be a potent inhibitor of cell growth and has been approved for phase II clinical investigation for treatment of secondary brain tumours in China. Nevertheless, the molecular mechanisms of β-elemene-induced cell death are not well established. It has become clear during the past decade that aberrations in the initiation or execution of the apoptotic programme at various levels are associated with most human malignancies, including prostate cancer, underscoring the importance of pharmacological therapies that target apoptosis regulators. Indeed, most chemotherapeutic agents act via the induction of apoptosis.[18-19]

In the current study, β-elemene induced apoptosis in prostate cancer cells, as demonstrated by flow cytometry, ELISA-based assay and TUNEL assay. These results indicate that β-elemene inhibits DU145 and PC-3 cell growth and triggers cell death by inducing apoptosis, suggesting the potential of β-elemene as an effective agent for the treatment of castration-resistant prostate cancer. Survival pathways for prostate cancer cell growth include deregulated expression and/or sequence variations of the PTEN gene that occur with high frequency in advanced prostate cancer, leading to aberrant action of Akt kinase activity, which in turn promotes tumour growth.[20] Loss of PTEN also permits activated Akt to phosphorylate the intracellular protein Bad, resulting in the release of the anti-apoptotic protein Bcl-2, which then leads to cancer cell survival.[20] Thus, recent therapeutic strategies have been directed at suppressing the anti-apoptotic function of Bcl-2.[46–48] The most successful targeting of the Bcl-2 axis in prostate cancer, however, has been achieved using chemotherapy. For instance, success of docetaxel chemotherapy in prolonging survival of patients with castration-resistant prostate cancer was first demonstrated in 2004. Docetaxel is a taxoid that inhibits the depolymerisation of microtubules, leading to disruption of the mitotic process and cell cycle arrest at the
G2/M phase and apoptosis. In addition to its apoptotic effect via microtubule stabilisation, docetaxel also induces apoptosis by inhibiting Bcl-2, a key mechanism for cancer cell survival achieved by the effect of PTEN sequence variations on Akt signalling. Androgen-insensitive cells may overexpress Bcl-2 independent of the PTEN–Akt signalling effects.[20] Docetaxel phosphorylates the serine residues of Bcl-2, resulting in its inactivation and consequent activation of the caspase cascade and apoptosis.

To understand the molecular mechanisms of β-elemene actions in prostate carcinoma cells, we investigated its effects on several apoptotic regulators. Bcl-2, a protein on the mitochondrial outer membrane, prevents apoptosis by suppressing the release of the caspase-activating protein cytochrome c from mitochondria.[21-22] Despite controversy regarding the role of Bcl-2 expression in the induction of apoptosis in prostate cancer cells, there is general agreement that Bcl-2 is involved in the mechanisms of prostate cancer initiation and progression, as well as the emergence of androgen-insensitive prostate cancer cells.[23-24] Furthermore, Bcl-2 expression has been shown to be associated with metastatic stages in prostate cancer.[25] Studies have also demonstrated that high Bcl-2 expression levels and low Bax expression levels are correlated with a poor therapeutic response of prostate cancer to radiotherapy,[26-27] indicating that Bcl-2 overexpression is an adverse prognostic indicator. In the present study, we demonstrated that β-elemene reduces the expression of Bcl-2 in a dose-dependent manner, suggesting that β-elemene induces apoptosis in human prostate cancer cells by modulating Bcl-2 expression.

To date, 14 distinct mammalian caspases have been identified and classified as initiator or effector caspases. The initiator caspases such as caspase-2, -8, -9 and -10 are activated by various apoptotic signals, after which they cleave and activate downstream effector caspases. In response to some death stimuli, cytochrome c is released from mitochondria into the cytosol, where it promotes the formation of a caspase-activating complex that includes cytochrome c, Apaf-1 and procaspase-9. This apoptosome complex triggers the activation of caspase-9, which leads to a proteolytic cascade that activates downstream effector caspases.[58,59] The effector caspases, which include caspase-3, -6 and -7, target specific cellular protein substrates for either activation or inactivation.[33,60] Accumulating evidence demonstrates that caspase cascades, especially via caspase-3, -7 and -9, are involved directly or indirectly in the execution of apoptosis in response to diverse stimuli in prostate cancer cells.[16,28-32] For example, caspase-3 cleaves and thereby inactivates PARP, which is generally considered to be an early marker of
chemotherapy-induced apoptosis.[33] The current study revealed that β-elemene treatment of prostate cancer cells resulted in the activation of caspase-3, -7, -9 and -10 and the cleavage of PARP, suggesting that β-elemene induces apoptotic cell death through a caspase-dependent mitochondrial pathway in prostate carcinoma cells.

Conclusions
In summary, we have demonstrated that β-elemene suppresses the growth and proliferation of prostate cancer cells and other types of tumour cells in vitro, indicating that β-elemene has broad-spectrum antitumour activity. We have also shown that β-elemene exerts its antitumour effect in prostate carcinoma cells by inducing apoptosis via a pathway involving the downregulation of Bcl-2 expression, enhancement of cytochrome c release, activation of caspase-3, -7, -9 and -10, and cleavage of PARP. Although the clinical treatment and management of castration-resistant prostate cancer remains a challenge, β-elemene holds promise as a new potentially effective therapeutic agent for prostate cancer and other types of solid tumours.

References:


β-elemene

Scientific Name
Ethenyl-1-methyl-2,4-bis(1-methyl ethenyl) cyclohexane
Clinical Summary
Beta-elemene is a volatile terpene found in botanicals such as celery, mint, and in many herbs used in traditional medicine. Whereas the purified form is generally not used as a dietary supplement because of poor absorption, many patients consume herbs high in beta-elemene in belief that they help cure cancer.

In vitro studies show anti-proliferative effects of beta-elemene in various cancer cells through cell-cycle arrest and induction of apoptosis (3) (4) (5) (16) (17) (19) (20). It also enhanced the activity of cisplatin against prostate cancer cells (18). The parenteral form of beta-elemene isolated from Rhizoma zedoariae, a type of ginger, has been studied in Asia and is reported to relieve pain, decrease the side effects of chemotherapy, and increase the quality of life in cancer patients. However, human trials conducted so far are of poor quality (1) (15). More studies are needed to understand the safety and efficacy of this product.

Food Sources
Rhizoma zedoariae (E Zhu)
Curcuma aromatica (Wenyujin)

Purported Uses
Cancer Treatment

Mechanism of Action
In an erythroleukemic cell line, beta-elemene inhibited telomerase activity, which was enhanced in combination with cyclophosphamide (9). It also demonstrated anti-fibrotic effects, inducing decreases in plasma angiotensin II levels and angiotensin II receptor type 1 expression, reducing collagen formation in a liver fibrosis rat model (10). Beta-elemene also exhibits anti-inflammatory effects, with reduction in LPS-induced nitric oxide production and prostaglandin E2 by rat peritoneal macrophages (11).

Beta-elemene has been shown to affect cancer cells via different mechanisms. In an in vitro study, following administration of beta-elemene, leukemia cells were arrested in S/G2 phase and underwent apoptosis (2). The antiproliferative effects were dependent on p38 MAPK activation/phosphorylation, cell cycle arrest in G0/G1, and inhibition of tumor growth of glioblastoma cells (3). Beta-elemene also induced G2/M arrest in lung carcinoma cells and ovarian cancer cells by combined reduction of cell-cycle promoters Cdc2 and cyclin B1, and elevated cell
cycle regulators p53 and p27 (4) (5). Derivatives of beta-elemene showed antiproliferative activity in human cervical carcinoma cells through a decrease in cell cycle protein Cyclin D1 and thus cell cycle suppression (14). Furthermore, beta-elemene may enhance the effects of chemotherapy and radiation. In non-small cell lung cancer cells, apoptosis was induced by increased expression of Bax and p-Bcl-2, decreased Bcl-2 and XIAP, and augmented cisplatin-induced increases in caspase activity (6). In another study, apoptosis response to the taxanes paclitaxel and docetaxel in lung carcinoma cells was enhanced with beta-elemene, through increases in cytochrome c, caspase activity, downregulation of Bcl-2, and altered cell membrane permeability (7). Beta-elemene combined with radiation induced apoptosis in lung adenocarcinoma cells, possibly by sensitizing cancer cells to radiation (8).

**Pharmacokinetics**

Beta-elemene is rapidly metabolized and excreted and has a short half-life, approximately half an hour. It was shown to be highly lipophilic, capable of crossing the blood-brain barrier, and reached peak levels in target tissues at 15 minutes with high concentrations found in heart, kidney, liver, brain, and lung. In rats, beta-elemene is bound extensively to plasma protein, and less than 2% of the original compound was recovered in bile, urine, and feces demonstrating that the compound is metabolized rapidly in the body (12).

Modified beta-elemene compounds have been synthesized to increase bioavailability while maintaining anti-tumor activity. Adding a hydrophilic group demonstrated increased solubility and antiproliferative activity in human cervix epithelioid carcinoma HeLa, gastric carcinoma and leukemia cells (13).

**Herb-Drug Interactions**

Chemotherapy: Beta-elemene may increase the activity of cisplatin and taxanes (6) (7).

Radiation: Beta-elemene may increase the effects of radiation (8).

**Literature Summary and Critique**

A review of randomized clinical trials of beta-elemene conducted between 1996 and 2005 shows that the trials are of poor methodology, with little allocation concealment and lack of statistical analysis (1) (15). Well designed studies are needed to determine the anticancer effects of beta-elemene.

**References**


11. Lim SS, Shin KH, Ban HS, et al. Effect of the essential oil from the


