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Review

Recent advances in the study of elemene on cancer

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Elemene, a natural sesquiterpene, has a wide antineoplastic spectrum, including multidrug-resistant tumors. Here, we summarize the literature about elemene's effects on cancer. Elemene has antiproliferative activity, blocks cell cycle, induces apoptosis, inhibits angiogenesis, reverses drug resistance, enhances radiotherapy and chemotherapy and improves immunity of cancer sufferers. Besides, more active *β*-elemene derivatives have been designed and synthesized. Some of them have better water solubility or antitumor activity. But those derivatives' anticancer effects need more experiments to test for both safety and efficacy.

Key words: Elemene, anticancer, Antiproliferative activity

INTRODUCTION

Elemene (1-methyl-1-vinyl-2, 4-diisopropenyl-cyclohexane, $C_{15}H_{24}$) is a natural sesquiterpene extracted from *Curcuma wenyujin*. Elemene has three isomers, *β*-, *γ*- and -, with *β*-elemene as the main component and the other two as the minor. Numerous reports have appeared in Chinese scientific literature since Guo et al. (1983) isolated *β*-, *γ*- and *δ*-elemene from *C. wenyujin*. In 1993, elemene injection was applied clinically as a national second-class new drug of anti-tumor in China. *β*-elemene is the main ingredient of elemene injection, and that also contains a small amount of *γ*- and *δ*-elemene and other terpenoids. The active pharmaceutical ingredients of elemene include *β*-elemene as the main ingredient, *γ*- and *δ*-elemene and *β*-caryophyllene as one main impurity (Sun et al., 2009). *β*-, *γ*- and *δ*-elemene account for more than 85% in elemene injection. The major advantages of elemene as an anticancer drug are that (a) it is non-cytotoxic and therefore can be well tolerated and accepted by cancer patients, (b) it has a wide spectrum of antineoplastic activity, including drug-resistant tumors, (c) it can reverse the resistance to other drugs and does not lead to multidrug resistance, (d) lemene can pass through the blood-brain barrier and marrow-blood barrier, and (e) it can improve patients' life quality (Zou, 2001; Yang, 1999; Zhang, 2010) Elemene is associated with diverse therapeutic benefits to lung cancer, hepatocellular carcinoma, brain tumor, nasopharyngeal carcinoma, esophageal cancer, gastric cancer, bone metastasis, leukemia, multiple myeloma, and malignant pleural effusion. Elemene exhibits antiproliferative effect correlated to G2/M phase arrest, induces apoptosis by the Caspase cascade of the mitochondrial pathway, inhibits angiogenesis by down regulating Vascular endothelial growth factor (VEGF) and CD34, reverses drug resistance associated with down regulating B cell lymphoma/lewkmia-2 (Bcl-2), and show radiosensitization and chemosensitization when combined with radiation and other anti-cancer chemicals. Moreover, structural modifications of *β*-elemene have been designed to pursue better water solubility or more effective anti-tumor activity. Some of them have been examined for their antineoplastic effects.

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Elemene inhibits cell proliferation

The effect of elemene on inhibiting proliferation of tumors has been cited in several reports. Elemene exhibits antiproliferative effect on many cancer cell lines, including

non-small-cell lung cancer (NSCLC). Wang et al. (2005) used cell death detection ELISA Kit to assay human NSCLC H460 and A549 cells and human lung fibroblast CCD-19Lu and bronchial epithelial NL20 cells for inhibiting proliferation. The half maximal inhibitory concentration (IC_{50}) of *β*-elemene (98%) on H460 cell growth was at 50, 46 and 42 µg/ml and on A549 cells at 61, 53 and 48 µg/ml at 24, 48 and 72 h, respectively. The IC_{50} s were 108, 100 and 98 µg/ml for the control lung fibroblast CCD-19Lu cells, and 145, 132 and 126 µg/ml for bronchial epithelial NL20 cells at 24, 48 and 72 h, respectively. Zhang J et al 2008 found the IC_{50} of 2% *β*-elemene injection on human glioblastoma U251 cells was 0.062 g/L and 2% *β*-elemene injection significantly inhibited the expression of proliferating cell nuclear antigen (PCNA). PCNA is necessary for DNA replication, and plays an important role in cell proliferation and it a reliable indicator of cell proliferation. Gu et al. (2005) reported the inhibitory effect of *β*-elemene (99.8%) on tubulin polyrnerization, which was one of the mechanisms of elemene's antitumor activity.

The effectiveness of elemene injection in treating brain tumours was studied using the G-422 tumour cell model in Sprague–Dawley rats. When 80 mg/kg elemene emulsion was used, the best tumour-inhibiting rate and the best life-extending rate of elemene were 34.46 and 64.43%, respectively, which provided experimental evidence of the characteristic of elemene of passing through the blood-brain barrier (Wu et al., 2009). Yao et al. (2007) demonstrated the significant antiproliferative effect of *β*-elemene (98%) in a dose- and time-dependent manner on the brain glioblastoma cell line C6 through downregulating of phosphorylated extracellular signal-regulated kinase (ERK). ERK played a role in reversing cell death signals. Elemene inhibited the growth of laryngeal cancer cell line HEp-2 *in vitro* and *in vivo*. The cytotoxic effect of elemene injection on the cells was evaluated by Tao et al. (2006). Compared with control groups, elemene significantly inhibited the protein expression of eukaryotic initiation factors (eIF4E and eIF4G) (Tao et al., 2006). HEp-2 cell growth was significantly inhibited by elemene *in vitro* and the IC_{50} was 62.70 µg/ml at 24 h (Cao, 2007). In addition, Elemene could inhibit the expression of Ki67 *in vivo* which may be an important antitumor mechanism of elemene. Ki67, a nuclear antigen associated with proliferation, is recently the most studied and the most promising marker of proliferation (Cao, 2007). According to Shi et al. (2009), the half maximal inhibitory concentrations (IC_{50}s) of *β*-elemene (98%) on thyroid cancer cell lines IHH-4 and ASH-3 *in vitro* were at 42.2, 36.6 and 39.1 µg/ml, and 52.4, 37.1 and 29.1 µg/ml, at 24, 48 and 72 h, respectively. Li et al. 2010 investigated *in-vitro* the antiproliferation of *β*-elemene (98%) on solid tumor cell lines, including human prostate cancer cell lines DU145 and PC-3, brain glioblastoma cell lines A-172 and U-87MG, brain astrocytoma CCF-STTG1, colon carcinoma CCL-222 and CCL-225, lung adenosquamous carcinoma NCI-H596, small cell lung carcinoma NCI-H69, HeLa cervical adenocarcinoma CCL-2, cervical carcinoma ME-180 and HTB-33, breast adenocarcinoma MCF-7 and T47D. The IC_{50}s of *β*-elemene on these solid cell lines were at 75, 105, 65, 88, 82, 47, 67, 95, 52, 63, 68, 68, 93, 63 µg/ml at 24 h.

The data suggested that *β*-elemene exerted anticancer activity toward a broad spectrum of solid cancers. *β*-elemene also inhibited the proliferation of human multiple myeloma RPMI-8226 cells in a time- and dose-dependent manner (Chen et al., 2010). The antiproliferative effect of elemene on human gastric carcinoma SGC-7901 cells was investigated *in vitro* (Chen et al., 2008). The cell growth was inhibited and the mechanism was thought to associate with down regulation by elmene of peroxisome proliferators-activated receptor gamma (PPAR *γ*). In human salivary gland, adenocarcinoma SACC-83 cells treated with *β*-elemene, the expression level of eIF4E (a eukaryotic initiation factor) decreased in a dose- and time-dependent manner ($P<0.05$), which was a possible mechanism for *β*-elemene to show antiproliferative activity (Chen et al., 2008).

In HeLa cell, the effect of elemene on transcription factor ELK1 and its target gene was investigated. Elemene can inhibit HeLa cell proliferation, which might be related with suppression of c-fos gene through inhibiting expression of phosphorated ELK1. Transcription factor ELK1 is a proto-oncogene c-fos regulatory factor (Chen et al., 2008). eIF4E is involved in apoptosis and tumor angiogenesis. Down regulation of eIF4E inhibits cell growth. 0.5% Elemene injection also inhibited the expression of hnRNP A2/B1 mRNA and protein in A549 cells. HnRNP (heterogeneous nuclear ribonucleoprotein) is a group of RNA-binding proteins, including hnRNP A, hnRNP B and hnRNP C, etc., which is responsible for post-transcriptional regulation of gene expression. HnRNP A2 / B1 play a critical role in the process. This may be the direct reason for antiproliferative activity of elemene (Zhou et al, 2004). The broad spectrum anti-proliferation of elemene is associated with its potential to induce cell cycle arrest and apoptotic cell death.

ELEMENE REGULATES CELL CYCLE

Many studies so far indicate the effectiveness of elemene on arresting tumor cells at different stages. The progression of cell cycle is regulated by cyclins and cyclin dependent kinases (CDK) through the four phases of G1, S, G2, and M. Cyclins and CDKs drive the cell from one phase to the next while CDKs are used in post-cell-division settings as well as in transcriptional processes. Wang et al. (2005) found that *β*-elemene (98%) could arrest NSCLC cell lines (H460 and A549) at G2/M phase, and the arrest was accompanied by decreases of cyclin B1 and phospho-Cdc2 (Thr-161) and increases of $p27^{kip1}$

and phospho-Cdc2 (Tyr-15). CDK1 (Cdc2, a cyclin-dependent kinase) and cyclin B are the two major players for control of the G2-M transition. Shi et al 2009 suggested that the mechanism of elemene arrest thyroid cancer IHH-4 cell cycle in G1 was involved in downregulating cell cycle regulatory protein E, CDK2 and CDK6. In human chronic myelogenous leukemia K562 cells, Lu and Xiang 2009 reported that G1 arrest was associated with up regulation of $p21^{WAF1}$ (P21 wild-type p53 activated fragment), which suggested the principal transcriptional target of *β*-elemene is p53 in the context of the G1 checkpoint arrest. Inactivation of p53 gene (an important anti-oncogene) plays an important role in tumor formation. The effectiveness of *β*-elemene on human multiple myeloma cells RPMI-8226 was investigated *in vitro* (Chen et al., 2010). At the same time, *β*-elemene repressed telomerase activity of SGC-7901 cells. The repression was related to the down regulation of c-myc gene and Bcl-2 gene expressions. C-myc gene plays an important role in the regulation of telomerase activity. C-myc is a transcription factor encoded by a proto-oncogene myc and is involved in cell differentiation and proliferation. Its regulation of expression is closely related with the generation of tumor. C-myc up regulates human telomerase reverse transcriptase (hTERT) activates telomerase and then causes cancer. In another study, elemene down regulated hTERT in SGC-7901cells in a dose and time-dependent manner (Fan et al., 2001; Chen et al., 2006) investigated the effect of 2% *β*-elemene injection on telomerase activity and expression of human telomerase reverse transcripase (hTERT) in U251 cells. *β*-elemene decreased the expression of Bcl-2 and hTERT gene, and thus the telomerase activity was impaired too. The morphological changes of RPMI-8226 cells were studied and the proportion of RPMI-8226 cells in the Go/G1 phase increased, and that in the S and G2/M phases decreased. Elemene also showed remarkable growth inhibition on human hepatic cancer 7402 cells and cervix cancer HeLa cell by arresting cell cycle in G2/M phase (Sun et al 2009). To study the mechanism of elemene treating ascites hepatoma cell line Hca-F_{25}/CI-$16A_3$, retinoblastoma tumor suppressor protein (pRB) and adenovirus E2 promoter-binding factor-1 (E2F-1) were examined *in vivo*. The mechanism of elemene signify-cantly inhibiting effect on Hca-F_{25}/CI-$16A_3$ cells is relative to expression of pRB, but not so closely relative to E2F-1. E2F and pRB alone with cyclinD1, CDK4/CDK6 and p16 together drive the cell from G1 phase to S phase. This study revealed that over expression of pRB caused by elemene leaded to cell cycle arrest in G1/S and pRB is an effective target of elemene (Ren and Zuo, 2002). The telomerase activity was investigated in retinoblastoma HXO-RB_{44} cells and it was found that elemene injection suppressed telomerase activity in a dose-dependent manner (Wei et al., 2007). In the colon carcinoma Lovo cells, Elemene was shown not only to have a relatively strong inhibitory effect on the proliferation of Lovo cells by blocking the cell cycle in the G0/G1 phase. The telo-merase activity was also suppressed in a concentration and time-dependent manner (Huang et al., 2004).

ELEMENE INDUCES APOPTOSIS

Cancer cells always elude apoptosis by deregulating genes that perpetuate programmed cell death (apoptosis). Several studies documented elemene-mediated apoptosis by regulating multiple targets in the apoptotic machinery. Evidence for reduced cell viability has been observed in response to elemene treatment of breast, NSCLC, bone, leukemia, larynx, brain carcinomas, and pancreatic cancer cells. Some classical hallmarks of apoptosis such as DNA fragmentation, chromatin condensation, and translocation of phosphatidyl serine across plasma membrane have been reported too.

Wang et al. 2005 found that *β*-elemene (98%) activated Caspase (Cysteine aspartic acid specific protease) -3, -7 and -9, down regulated Bcl-2, induced cytochrome c release and up regulated the levels of cleaved Caspase-9 and poly (ADP-ribose) polymerase in NSCLC cells. These indicated that *β*-elemene may be through the cytochrome c-mediated apoptotic pathway to induce lung cancer cell death. Cao et al. (2008) found a down regulation of Bcl-2 and an up regulation of p53 in A549 cells when treated with elemene injection. On the contrary, Liu et al. (2001) reported that *β*-elemene inhibited the viability of human gastric cancer cell lines MGC803 and SGC7901 in a dose-dependent manner by inducing apoptosis and protective autophagy in the two cell lines. As the result indicated, autophagy in MGC803 and SGC7901 resulted in both survival and cell death. On the one hand, *β*-elemene induced apoptosis, which led to death; on the other hand, it activated a protective autophagy to adapt to the stressful conditions and protect cells from death. The mechanism leading to two opposite consequences was inhibition of PI3K/Akt/mTOR activity by *β*-elemene. PI3K, Akt and mTOR all played important roles in the inhibition of apoptosis. The expression of survivin was investigated in retinoblastoma HXO-RB_{44} cells and it was found that elemene injection down regulated the expression of survivin in a dose-dependent manner. In agreement with previous studies, the decrease in survivin expression correlated with an increase in apoptosis of HXO-RB_{44} cells (Wei et al., 2007). When elemene injection in conjunction with etoposide treated HXO-RB_{44} cells for 24 h, the inhibition effect on the expression of survivin was enhanced evidently (Wei et al., 2007). So far as we know, survivin is the most powerful inhibitor of apoptosis. Survivin inhibits the Caspases signaling pathway, like Caspase-3 and Caspase-7 to induce apoptosis. The effects of *β*-elemene on human gastric carcinoma SGC-7901 cells were conducted *in vitro* (Guan et al., 2003; Fan et al., 2001a, b, c, 2006; Wang and Qu, 2010). *β*-elemene induced

SGC-7901 cells apoptosis by down-regulating survivin expression and up regulating Caspase activity. At the same time, β-elemene repressed telomerase activity in SGC-7901 cells. The repression might be related to the down regulation of expression of c-myc gene and Bcl-2 gene. Another human gastric carcinoma cell line BAC823, β-elemene also induced apoptosis and phosphorylated p38MAPK (mitogen-activated protein kinase) and thus activated MAPK pathway. The MAPK pathway was involved in induction of apoptosis of BAC823 cells. As for glioblastoma multiforme, one of the deadliest human cancers, treatment of glioblastoma cell lines C6 and U251 with β-elemene (98%), led to phosphorylation of p38 MAPK and apoptosis of C6 and U251 cells. The MAPK pathway is one of critical targets in gliomagenesis that regulate cellular proliferation, cell scatter and migration. These findings indicated that the p38 MAPK pathway was critical for the antineoplastic activity of β-elemene (Yao et al., 2008). When glioblastoma cells U87 were treated with β-elemene (98%) at various doses or for different times, MKK3, MKK6 phosphorylated MKK3 (p-MKK3) and phosphorylated MKK6 (p-MKK6) were detected to reveal the anti-glioblastoma proliferation mechanism of β-elemene. Mitogen-activated protein kinase kinase-3 (MKK3) and -6 (MKK6) are two upstream kinases of p38 MAPK and can regulate cellular growth, fission, differentiation and apoptosis. MKK3/6 can be activated through phosphorylation. The result indicated that β-elemene up regulated phosphorylated MKK3 and phosphorylated MKK6, inhibited the proliferation of U87 and arrested them in G0/G1 phase. At the same time, inhibition of MKK3 and MKK6 reversed the antitumor effect of β-elemene. Furthermore, when one of MKK3 and MKK6 was inhibited, the other was compensatorily activated in the presence of β-elemene. These results implied that mutually compensatory activation of MKK3 and MKK6 mediated the anti-glioblastoma effect of β-elemene. MKK3 and MKK6 might be possible putative targets for glioblastoma therapy (Zhu et al., 2011). Another study revealed that β-elemene (98%) inhibited U87 cell viability through activating the GMF β signaling pathway. In contrast, inactivating GMF β, a human glia maturation factor, reversed the anti-glioblastoma effect of β-elemene and reduced the phosphorylation of MKK3/6. GMF β, an intracellular protein, is primarily localized in the mammalian central nervous system and plays an important role in regulating the growth and development of glial cells and neurons. The result suggests that GMF β signaling pathway also mediates the anti-glioblastoma effect of β-elemene. GMF β, an activator of MKK3/6, might be another target for molecular therapy against glioblastoma alone with MKK3 and MKK6 (Zhu et al., 2011).

Series of studies were done on human bladder carcinoma BIU-87 cells. β-elemene changed the nuclear chromatin of the experimental group, increased intracellular free Ca^{2+} in a dose-dependent manner, decreased cell mobility and Bcl-2 expression also in a dose-dependent manner and induced apoptosis (Li et al., 1999, 2007; Shi et al., 2009) detected the expression of Bcl-2 and Caspases-3, Caspases-8 and Caspases-9 proteins in thyroid cancer cell line IHH-4 treated by β-elemene (98%) *in vitro* and shown that β-elemene up regulated Caspase-3 and down regulated Bcl-2, but β-elemene has no direct effect on Caspase-8 and Caspase-9. Bcl-2 inhibits the release of cytochrome c from mitochondrion, thus inhibits apoptosis. Caspase 8 and 9 initiate apoptosis. β-elemene and down regulated survivin in a dose- and time-dependent manner in K562 cells (Lu and Xiang, 2008; Li et al., 2010) investigated *in-vitro* that β-elemene (98%) induced apoptosis on human prostate cancer cell DU145 and PC-3. The possible pathway of inducing apoptosis on both DU145 and PC-3 was involved in down regulating Bcl-2 expression, enhancing cytochrome c release, activating Caspase-3, -7, -9 and -10, and cleaving PARP [poly (ADP-ribose) polymerase]. Caspase-3 cleaves and thereby inactivates PARP, which is considered to be an early marker of chemotherapy-induced apoptosis.

ELEMENE INHIBITS ANGIOGENESIS

Angiogenesis supplies oxygen and nutrients to sustain tumor growth. CD34 is an important molecular marker for angiogenesis and vascular endothelial growth factor (VEGF) an initiator of angiogenesis and controlling the progress of tumor growth and metastasis. Elemene has direct inhibitory effects on CD34 and VEGF. Gu et al. 2005 investigated the antiangiogenic effect of β-elemene (97%) on B16F10 melanoma cells *in vitro* and *in vivo*. The result clearly demonstrated that β-elemene inhibited expressions of CD34 and VEGF. The VEGF-mediated antiangiogenesis mechanism was through targeting VEGF or competitively binding VEGF receptors or affecting its upstream or downstream signaling pathway, which remains more work to identify it. Elemene inhibited the growth of laryngeal cancer cell line HEp-2 *in vitro* and *in vivo*. The effects of elemene injection on the cells were evaluated by Tao et al. (2006). Compared with control groups, elemene significantly inhibited the expression VEGF and decreased the micro-vessel density (MVD), which ascribed to enhancing Caspase-3 activity (Tao et al., 2006). In addition, Elemene could inhibit the expression of VEGF-C, vascular endothelial growth factor receptor-3 (VEGFR-3). MVD is an important indicator of angiogenesis. VEGF-C binding with VEGFR-3 regulates tumor angiogenesis and lymphangiogenesis, and promotes tumor cells metaptosis into the lymphatic vessels (Cao, 2007). In a nude mice model transplanted orthotopically with human gastric cancer BGC823 cells, after treated with 80 mg/kg.d elemene injection two times a week for three weeks, the MVD and the expressions of VEGF and p53 protein were detected. The MVD, VEGF

mRNA level and the expressions of VEGF and p53 protein were all significantly lower in experimental groups (Li et al., 2010; Lei et al., 2008) evaluated the effect of elemene injection on the expression of heparanase (HPA) in BGC823 cells. Elemene reduced the HPA expression level significantly in a dose- and time-dependent manner. HPA can promote tumor angiogenesis, and is involved in metastasis. Thus elemene prevented from metastasis via lymphatic or angiogenesis by reducing HPA expression in BGC823. In human salivary gland adenocarcinoma SACC-83 cells, when compared to cisplatin, elemene not only significantly inhibited the expression of eIF4E but also that of VEGF (Liu et al., 2011; Li, 2009) reported that elemene injection inhibited the activity of MMP-2 and MMP-9 in human vascular endothelial ECV-304 cells. MMP-2 and MMP-9 are two mine matrix metallo-proteinases which can degrade extracellular matrix and structural membrane proteins. Inhibition of MMP-2 and MMP-9 can effectively prevent the vascular basement membrane from degradation, and reduce angiogenesis.

ELEMENE REVERSES DRUG RESISTANCE

Drug resistance plays an important role in protecting cancer cells from chemical attack. Elemene reverses drug resistance either used only or applied in conjunction with cisplatin or other anticancer drugs. Drug resistance is associated to multidrug resistance (MDR) gene and multidrug resistance protein (MRP). P-glycoprotein (P-gp) is the product of MDR1 gene and P-gp is mainly responsible for multidrug resistance. P-gp is an energy-dependent unidirectional drug pump, and the drug may be excreted out the cell, which results in reduction of intracellular drug accumulation. MRP helps tumor cells to resist antitumor drugs along with P-gp. Besides the over expression of Glutathione S-transferase-π (GST-π) contribute to drug resistance.

Li et al. (2005) show that treatment of the cisplatin-resistant human ovarian carcinoma cell line A2780/CP with *β*-elemene (98%) and cisplatin, down regulated cyclin B1 and Cdc2 expression, but elevated the levels of p53, $p21^{waf1/cip1}$, $p27^{kip1}$ and Gadd45. This demonstrated for the first time that *β*-elemene enhanced cisplatin-induced growth inhibition in A2780/CP by modulating the cell cycle G2 checkpoint and inducing cell cycle G2-M arrest. Wang et al. (1996) used human hepatoma cell line BEL-7402 to develop its multi-drug resistant strains BEI-7402/DOX. BEI-7402/DOX was evidently resistant to mitomycin-e, epirubicin, daunorubicin, vincristine and harringtonine but not to elemene emulsion. The expression of P-gp and MDR1 were detected and it was found that both of them were significantly higher in BEI-7402/DOX cells treated with mitomycin-e, epirubicin, daunorubicin, vincristine and harringtonine than those treated with elemene injection. This demonstrated that resistant tumor cells remained sensitive to elemene.

Elemene could reverse the multidrug resistance (MDR) of SGC7901/VCR to vincristine and adriamycin but did not work as to 5-fluorouracil and cisplatin. The possible reason was not only down regulation of P-gp in SGC7901/VCR cells but also that of MRP (Wang et al., 2005; Chen et al., 2006) found that elemene injection increased evidently the intracellular accumulation of AMD in U251/AMD cells, reduced the IC_{50} of U251/ADM cells from 0.915 to 0.051 mg/L and significantly down regulated MDR1, MRP and GST- π in U251/ADM cells. Gao et al. (2006) investigated the effect of elemene on the expression of MDR1 in mouse hepatocarcinma Hca-A2/p cells and found that the innate expression of MDR1 in Hca-A2/p was 92.9% and innate expression of MDR1 increased in Hca-A2/p cells treated with chemotherapy. Elemene reversed MDR1 gene and was effective in killing tumor by itself and more effective by combining with mitomycinum c. Yang et al 2006 cultured a paclitaxel resistant human lung adenocarcinoma cell line SPC-A1/Taxol, investigated its resistance to elemene and found that SPC-A1/Taxol was still sensitive to elemene injection. Elemene injection in K562/ADM cells decreased the expression of Bcl-2 from 94.8 to 66.7% and that of P-gp from 98.7 to 76.8% (Hao et al., 2005). Elemene injection (4 μg/ml) in conjunction with tetramethylpyrazine (350 μg/ml) apparently improved antiproliferative effect on K562/ADM cells and the resistance-reversing was 4.65-fold (Hao et al., 2002). As for human breast cancer MCF-7/ ADM cells, when elemene injection was 6 and 13 μg/ml, the resistance-reversing was 1.4- and 2.2-fold, respectively (Hu et al., 2004). But when 6 mg/L elemene injection was combined with 15 mg/L realgar, the resistance-reversing was improved to 4.2-fold (Hu et al., 2007). The multi-resistance to ADM of human osteosacoma cell MG-63/ADM was reversed by elemene, GST-π protein down regulated and the intracellular accumulation of ADM increased (Huang, 2009).

ELEMENE AND RADIOTHERAPY OF CANCER

Radiotherapy, also called radiation therapy, is the use of high energy rays to kill cancer cells. But it kills normal cells at the same time which causes severe side effects. It may be used to cure some cancers. Several experiments demonstrated that elemene sensitizes tumors to radiotherapy.

Jiang et al. (2009) firstly studied the radiosensitization *in vitro* on lung adenocarcinoma cell line A549 using elemene injection, and found that the reproduction of the group under radiation and *β*-elemene was significantly inhibited. The mechanism might be relevant to p53 up regulation and Bcl-2 down regulation. Fan et al. (2011) studied it in a nude mice model transplanted A549. The expressions of VEGF and CD34 were found a significant decrease in the compared to the control groups. The result definitely demonstrated that *β*-elemene was good

at radiosensitization. As for human tongue squamous cell carcinoma cell line Tca-8113, the chemosensitizing effect of β-elemene to conventional irradiation both *in vitro* and *in vivo* was studied. *In vitro* studies revealed that preexposure of cells with β-elemene for 24 h followed by exposure to radiation for 1 min resulted in 10 to 18.5% growth inhibition compared to 0.1 to 0.75% when radiation was used alone. The possible mechanism was that β-elemene induced apoptosis, down regulated Bcl-2, up regulated Bax and arrested cell cycle in G2/M (Wu et al., 2009). In nude mice transplanted human squamous cell carcinoma of tongue Tca-8113 model, β-elemene has synergistic effect with radiation to inhibit cell growth significantly differently from radiation alone (Wu et al., 2010). *In vitro* low-dosage β-elemene (10 mg/kg) followed by gradient radiation from 1 Gy to 5Gy can all enhance the effect of radiation on rabbit VX2 renal carcinoma (She et al., 2006). The expressions of Caspase-3 and Bcl-2 were detected. β-elemene up regulated Bax, down regulated Bcl-2 protein expressions and promoted apoptosis through Caspase-3 pathway. Chen et al 2010 used elemene injection combined with radiotherapy to treat malignant glioma clinically and the 1-, 3-year survival rates were promoted to 64.7 and 41.2% from 42.3 and 15.4%, respectively. Zhang et al 2010 used elemene injection combined with radiotherapy to treat metastatic bone cancer clinically and it released the pain and improved life quality of patients. Zhou et al 2010 used elemene injection combined with radiotherapy to treat lung cancer with brain metastasis clinically and the median survival time was improved from 10.9 to 15.0 months and it caused less leukocyte reduction, which meant that elemene relieved myelosuppression.

ELEMENE AND CHEMOTHERAPY OF CANCER

In several studies, elemene combined with other chemical anticancer drugs has synergistic inhibitory effect, can relieve the side effects of chemotherapy and improve patients' life quality. Besides, elemene can enhance the chemosensitization when applied in conjunction with antitumor drugs like cisplatin. When β-elemene (60 μg/ml) was combined with cisplatin (20 μg/ml) for 24 h to treat U87 glioblastoma cells, the cell growth inhibitory rate in the combination group was significantly higher than that in the individual cisplatin ($p<0.01$) and β-elemene ($p<0.01$) groups, which suggested that β-elemene increased the sensitivity of U87 to cisplatin-induced cytotoxicity .

Li et al. (2010) evaluated proliferation of the androgen-independent prostate carcinoma cell lines DU145 and PC-3 following a combination of β-elemene (98%) and cisplatin. Their results revealed reduction in cell prolife-ration, activation of the cisplatin-induced Caspase-3/7/10 and Caspase-9, cleavage of Caspase-3 and -9, inhibition of Bcl-2 and Bcl-X_L expression, and release of cytochrome c from mitochondria into the cells, which indicated that the combined use of β-elemene and cisplatin might be an effective treatment against the androgen-independent prostate carcinoma. Zheng et al. (2009) explored the effect of elemene combined with aclarubicin on the induction of human leukemia HL-60 cells apoptosis. Preexposure of HL-60 cells with elemene (10, 20, 40 μg/ml) for 2 h followed by exposure to aclarubicin (0.1 μg/ml) for 18 h resulted in 8.97 to 34.90% growth inhibition compared to 8.07% when 40 μg/ml elemene was used alone and down regulation of COX-2, NF-kappaB and PGE_2 expressions. Cao et al. (2010) found that Astragaloside and β-elemene inhibited growth of SGC7901 cell in a dose-dependent manner and down-regulated expression of COX-2, VEGF and PGE_2. Those results indicated that the anti-tumor mechanism of β-elemene combined with aclarubicin or Astragaloside was associated with inhibition of NF-kappaB and COX-2 expression, which caused the suppression of its down-stream product PGE_2 expression and down-regulation of VEGF, therefore induced apoptosis and decreased tumor growth. Qin et al. (2010) reported that elemene injection in conjunction with Oxaliplatin, Calcium folinate and 5-Fluorouraci significantly increased CD3, CD4 and CD4/ CD8 when used clinically to treat progressive gastric cancer, and the patients' immunity and life quality were improved. Elemene injection in conjunction with capecitabine and oxaliplatin regimen chemotherapy clinically had the same effect on progressive gastric cancer.

Gu et al. (2011) and Ma et al. (2008) investigated the synergistic inhibitory effect of elemene and tamoxifen on breast cancer MCF-7 cells and found that the inhibitory rates increased significantly and Bcl-2 and pS2 decreased significantly in MCF-7 cells exposed to elemene and tamoxifen. Bai et al. (2010) reported that β-elemene combined with curcumin has the synergistic inhibitory effect on inhibition and induction of apoptosis of adenoid cystic carcinoma SACC-LM cells. Zhang et al. (2008) reported that in Lewis lung carcinoma mouse, exposure to elemene injection (100 mg/kg, once a day) and paclitexal (1.2 μg/ml, three times a week) for two weeks resulted in significant difference in the inhibition rates, micro vessel density, and expressions of VEGF receptor 1 and 2 compared to control groups. Elemene injection and paclitexal have synergistic inhibitory effect on inhibiting angiogenesis via reducing the levels of VEGF and inhibiting the expression of VEGF receptors. While exposure to elemene injection (25 μg/ml) and paclitexal (25 μg/ml) for 24 h resulted in arresting cell cycle in G1 phase and appearance of apoptotic body in human salivary SACC-83 cells (Bai and Zhang, 2004). Tian et al. (2009) reported that elemene injection combined with paclitexal increased CD3+, CD4+ cells and the ratio of CD4+/CD8+ significantly when treating patients with advanced non-small cell lung cancer (NSCLC) clinically.

Elemene enhances immunity of cancer sufferers

Elemene was firstly used to investigate its effect on immunological function of macrophage. J447A.1 cells, the mouse macrophage strain, was exposed to Astragaloside IV and *β*-elemene *in vitro*. Following the treatment of Astragaloside IV and *β*-elemene *in vitro*, the levels of MHC_1, CD40, and CD86 on J447A.1 cell surface were significantly higher than those in the control group. After treatment of both Astragaloside IV and *β*-elemene the phagocytic function of macrophages for neutral red dye was obviously higher than that in control group ($P< 0.05$) with the increase of drug concentration and reactive time. Based on the result, it could conclude that *β*-elemene as well as the combination with Astragaloside IV could enhance the level of MHC molecules and co-stimulatory factors on the surface of macrophages and elevate the phagocytic ability of macrophages (Li et al., 2011). When patients with digestive tractneoplasm were treated with virus/*β*-elemene modified tumor vaccine after operation, then were detected the change of CD4+, CD8+, CD4+/CD8+ and IL-2, IFN-*γ*, IL-4 and IL-10 before operation and after immunization. After treatment, the percentage of CD4+, CD8+ or CD4+/CD8+ and all cytokines were significantly changed ($P<0.05$). Wang et al. (2008) and Tian et al. (2009) reported that elemene emulsion in combination with chemotherapy could significantly lower in patients with advanced non-small cell lung cancer, the CD3 +, CD4 + cells, CD4 + /CD8+ ratio, increase CD8 + cells, thereby enhance the immunity and life quality of patients. In mice transplanted cervical cancer cells U14, after treated elemene for 15 days the T cell sub-group, CD3+, CD4+ and CD8+ were significantly higher in agreement with previous studies (Wu et al., 2009). A human hepatoma cell line $HepG_2$ was used to demonstrate the increase in tumor cell immunogenicity induced by elemene in the level of gene. 24 genes were found to alter expression in $HepG_2$ cells treated with elemene. Based on this, people can develop new and effective cancer vaccine (Gao et al., 2002).

ELEMENE DERIVATIVES

Although elemene has such good antitumor activity, it also has limited bioavailability for poor water solubility, short half lives and rapid clearance from the body. So structural modifications of *β*-elemene have been designed hunting for better water solubility and better anti-tumor activity derivatives. Zhang et al. (2010) already summarized some works; here we review the latest progress in *β*-elemene derivatives.

Yu et al. (2008) synthesized *N*-(*β*-elemene -13-yl) tryptophan methyl ester for improving its antitumor activity. The apoptotic effect and the mechanism of *N*-(*β*-elemene-13-yl) tryptophan methyl ester were investigated in human leukemia HL-60 and NB4 cells. *N*-(*β*-elemene –13-yl) tryptophan methyl ester induced apoptosis in HL-60 and NB4 cells at concentrations less than 40 *μ*M and the mechanism was associated with the enhanced production of hydrogen peroxide (H_2O_2), the decrease of mitochondrial membrane potential, and the activation of Caspase-3 and the PARP [poly (ADP-ribose) polymerase] cleavage but not influencing the levels of Bcl-2 and Bax protein. These data suggested that this *β*-elemene derivative induced apoptosis in HL-60 and NB4 cells through a H_2O_2-dependent pathway. Sun et al. (2009) synthesized *β*-elemene monosubstituted amine, ether and rhenium coordinated complex. They got the pure *β*-elemene monosubstitued amine derivatives, ether derivatives and rhenium derivatives and tested *in vitro* the compounds' antiproliferative activities in human cervix epitheloid carcinoma HeLa cells. The IC_{50} values of monosubstitued amine derivatives and rhenium derivatives were significantly lower than parent *β*-elemene while those of ether derivatives were not. But all synthesized derivatives could reduce the cell survival checkpoint protein p-Rb while the protein level of Rb did not decrease and the cell cycle protein Cyclin D1 expression and arrest the cell cycle at G1 phase. Yu et al. (2011) synthesized five piperazine derivatives, 13-(3-methyl-1-piperazinyl)-*β*-elemene (1), 13-(cis-3,5-dimethyl-1-piperazinyl)- *β*-elemene (2), 13-(4-ethyl-1-piperazinyl)-*β*-elemene (3), 13-(4-isopropyl-1-piperazinyl)- *β*-elemene (4) and 13-piperazinyl- *β*-elemene (5). The five novel compounds' antiproliferative and apoptotic effects were determined in human leukemia HL-60, NB4, K562 and HP100-1 cells. Compound 1, 2 and 5 were more effective on inhibiting cell growth and inducing apoptosis than compound 3 and 4. The IC_{50} values of these compounds substituted with a piperazine were less than 10 *μ*M. The apoptosis activity of compound 1 was associated with the production of H_2O_2 as *N*-(*β*-elemene -13-yl) tryptophan methyl ester did (Yu et al., 2008; Ren et al 2009) synthesized three novel Re $(CO)_3$-*β*-elemene derivatives. The IC_{50} values of these derivatives were very low compared to *β*-elemene. Table 1 show multieffects of elemene.

CONCLUSIONS

Elemene's anticancer effect is already well known, but it's clear mechanism of antitumor is still to be explored. For elemene's poor water solubility, short half lives and rapid clearance from the body, many derivatives have been synthesized to pursue better water solubility and better bioavailability. Novel elemene formulations are being developed too, such as nanoparticles or liposomes as elemene carrier, elemene microemulsion, oral elemene o/w microemulsion. Liposomes as carriers are tumor-targeted, phospholipid bilayer vesicles wrapping water-soluble or fat-soluble drugs and can pass through the blood-brain barrier. Nanoparticles or nano-liopsomes as

Table 1. Multieffects of elemene.

Elemene's effects	Excitation	Inhibitory action
Antiproliferation		PCNA, tubulin polyrnerization, ERK, eIF4E, eIF4G, Ki67, PPARγ, ELK1, hnRNP A2/B1
Apoptosis	Caspase-3,-7,-9and-10,poly (ADP-ribose) polymerase, MKK-3 and-6; Releasing cytochromec	Bcl-2, Survivin, p38MAPK, GMFβ, PARP
Antiangiogenesis		CD34, VEGF, VEGFR-3, p53, HPA, MMP-2 and -9
Drug resistance		MRP, P-gp, MDR1, GST-π
Enhancement of immunity	MHC,CD3,CD4,CD4/CD8ratio,CD3+cells,CD4+cells, CD4+/CD8+ ratio	
Regulation of cell cycle	$p27^{kip1}$, $p21^{WAF1}$, pRB	cyclin B1, p53, telomerase activity, hTERT
Radiosensitization	Bax; Causing less leukocyte reduction	
Chemosensitization		Bcl-X_L, COX-2, NF-kappaB and PGE_2

carriers are tumor-targeted, and can release drugs into tumor organism in constancy. Elemene microemulsion has improved bioavailability and excellent phase stability and oral elemene microemulsion with excellent clarity, high entrapment efficiency, good patient compliance, and improved bioavailability has been designed and produced (Zhu et al., 2008; Wang et al., 2008; Zeng et al., 2010; Hu et al.,2011). As the progress not only in elemene's clear mechanism of antitumor but also in its better water solubility, bioavailability and novel carrier systems, elemene or its derivatives would contribute more to the fight against cancer for people.

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Abbreviations: PCNA, Proliferating cell nuclear antigen; **ERK,** extracellular signal-regulated kinase; **eIF4E and eIF4G,** eukaryotic initiation factors; **PPARγ,** peroxisome proliferators- activated receptor gamma; **ELK1,** E twenty-six (ETS)-like transcription factor 1; **hnRNP,** heterogeneous nuclear ribonucleoprotein; **CDK,** cyclin dependent kinases; **hTERT,** human telomerase reverse transcriptase; **pRB,** retinoblastoma tumor suppressor protein; **Caspase,** cysteine aspartic acid specific protease; **Bcl-2,** B-cell non-Hodgkin lymphoma; **p38MAPK,** p38 mitogen-activated protein kinase; **MKK,** mitogen-activated protein kinase-kinase; **GMFβ,** human glia maturation factor; **PARP,** poly (ADP-ribose) polymerase; **VEGF,** vascular endothelial growth factor; **VEGFR-3,** vascular endothelial growth factor receptor-3; **HPA,** heparanase; **MMP,** matrix metalloproteinase; **MRP,** multidrug resistance protein; **P-gp,** p-glycoprotein; **MDR1,** multidrug resistance1 gene; **GST-π,** glutathione S-transferase π; **Bax,** Bcl-2-associated x protein; **Bcl-X_L,** human Bcl Extra Large; **COX-2,** cyclooxygenase-2; **NF-kappaB,** nuclear factor kappa-light-chain-enhancer of activated B cells; **PGE_2,** prostaglandin E-2; **MHC,** major histocompatibility complex.

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