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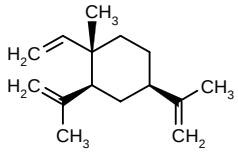
Elemene

Antineoplastic

β-Elemene

(1α,2β,4β)-2,4-Diisopropenyl-1-methyl-1-vinylcyclohexane
(1α,2β,4β)-1-Ethenyl-1-methyl-2,4-bis(1-methylethenyl)cyclohexane



$C_{15}H_{24}$ Mol wt: 204.35

CAS: 033880-83-0
CAS: 100762-52-5 (as racemic)
CAS: 192460-89-2 [as (–)-α-isomer]
CAS: 057172-50-6 (as racemic γ-isomer)
CAS: 030824-67-0 [as (+)-γ-isomer]
CAS: 029873-99-2 [as (–)-γ-isomer]
CAS: 020307-84-0 (as δ-isomer)
CAS: 013833-25-5 [as (+)-β-isomer]
CAS: 011029-06-4 (undefined isomer)
CAS: 005951-67-7 [as (+)-α-isomer]
CAS: 000515-13-9 [as (–)-β-isomer]

EN: 257947

Introduction

Patients with malignant disease generally fall into one of two categories: those with localized tumors who can be readily treated by surgery or radiotherapy with resulting long-term remission and cure, and another larger group of patients who present with disseminated tumors. In the latter case, treatment requires a systemic approach which may involve administration of chemical compounds. Although many chemotherapeutic regimens can be very effective in treating malignant disease, their lack of specificity can result in unacceptable toxicities. The problem of specificity of treatment, therefore, is a significant factor in the search for more effective therapies for malignant disease. China is famous for its rich resources of plants and animals. In recent years, the discoveries of natural products with high specificity have provided encouraging results for the treatment of tumors in China (1). For example, elemene, a new antitumor agent of plant origin with high specificity, has recently been discovered and recommended for clinical trials in China. This novel antineoplastic natural product has been shown to have a substantial clinical effect in the treatment of various tumors. Moreover, experimental and clinical data indicate that elemene has fewer side effects, especially no myelosuppression. These characteristics are significantly different from those of the conventional chemotherapeutic drugs.

Chemistry

Elemene is a naturally occurring compound that can be isolated from the traditional Chinese medicinal herb *Rhizoma zedoariae* native to south China, which was used to treat tumors in Chinese folk medicine (2).

Elemene exists as a mixture of α-, β-, γ- and δ-isomers. Pure isomers of elemene have been isolated and purified from the mixture. It has been demonstrated that the main antitumor active component of elemene is the β-isomer. Accordingly, elemene used in experimental and clinical investigations is also mainly composed of the β-isomer (3).

Antitumor Activity

Experimental studies have shown that elemene exerts obvious antitumor activity *in vitro* and *in vivo*. The cytotoxicity of elemene has been determined in various tumor cell lines *in vitro* (3-9). As shown in Table I, elemene inhibits the growth of uterocervical carcinoma HeLa cells, promyelocytic leukemia HL-60 cells, erythroleukemia K562 cells and drug-sensitive and doxorubicin-resistant hepatoma BEL-7402 cells. In addition, the growth of other tumor cells such as several pulmonary carcinoma cell lines (Lax, Anip-937, SPC-A1, H128, SPC) was also inhibited by the agent. However, the IC_{50} of elemene for normal human peripheral blood leukocytes is 254.3 mg/l. Based on the comparison of the IC_{50} between tumor cells and normal leukocytes, it appears that the growth inhibitory effect of elemene on tumor cells is much stronger than on normal cells. When elemene was used in combination with adriamycin or cisplatin *in vitro*, a synergistic inhibition of the growth of gastroadenocarcinoma SGC-7901 cells was found (10).

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Table I: Effect of elemene on cell growth in vitro.

Cells	IC_{50} (mg/l)
Uterocervical carcinoma HeLa	37.3
Promyelocytic leukemia HL-60	27.5
Erythroleukemia K562	81.0
Hepatoma BEL-7404 (dryg-sensitive)	48.9
Hepatoma BEL-7404 (drug-resistant)	51.3
Pulmonary carcinoma LAX	21.0
Pulmonary carcinoma Anip-937	30.1
Pulmonary carcinoma SPC-A1	23.8
Pulmonary carcinoma A549	29.1
Pulmonary carcinoma H128	36.6
Pulmonary carcinoma SPC	41.9
Normal peripheral blood leukocytes	254.3

Elemene was given i.p. to mice bearing Ehrlich ascites carcinoma EAC) and ascites sarcoma 180; the antitumor activities were significant (Table II). The drug also exhibited antitumor activities for other mouse ascites tumors such as sarcoma 37, leukemia L1210, leukemia P388, reticulosarcoma ARS, and rat ascites tumors such as Yoshida sarcoma. Antitumor activities of elemene were also found in several subcutaneously implanted solid tumors such as mouse Lewis lung carcinoma and rat Walker 256 sarcoma (Table III) (11).

Mechanism of Action

It has been shown that the mechanism of action of elemene involves direct cytotoxic activities as well as indirect immunostimulatory effects.

Direct cytotoxic activities

It was found by electron microscope technique that elemene selectively decreased microvilli of the surface of tumor cells, suggesting that the drug may affect some characteristics of tumor cell membrane. Further studies indicated that elemene suppressed DNA, RNA and protein syntheses in EAC cells and several lung cancer cells, which may also contribute to its cytotoxic effect (11). The effect of elemene on the cell-cycle progression of tumor cells was studied *in vitro* by using flow cytometry technique. When HL-60 cells were treated with 20 mg/l of elemene, cell proportion at the G_2M phase was decreased and at the S stage was increased (Table IV). These results suggest that the inhibitory effects of elemene on tumor cell growth are related to cell cycle arrest from S to G_2M phase transition, consequently inhibiting the mitosis of tumor cells (8).

Although targeting of apoptosis is a relatively novel concept, there is sufficient evidence to indicate that induction of apoptosis could provide a highly specific means of attacking malignant cells (12, 13). Induction of tumor cell apoptosis by elemene has recently been confirmed (8). When HL-60 cells were treated with 20 mg/l elemene, flow cytometry analysis showed that the histogram exhibited the distinct apoptotic feature of sub-G_1 peak (apoptosis peak). The percentages of apoptotic cells with elemene treatment of 4, 24 and 48 h were 41.5, 35.3 and 47.7%, respectively. An important hallmark of apoptotic cell death is the fragmentation of genomic DNA into integer multiples of 180 bp units producing a characteristic ladder on agarose gel electrophoresis. To characterize elemene-inducing apoptotic cell death in HL-60 cells, internucleosomal DNA fragmentation was analyzed after the cells were exposed to different concentrations of elemene from 2-24 h. The distinct internucleosomal DNA fragmentation ladder was observed in HL-60 cells treated for 2 h. Moreover, in comparison with 2-h treatment, the concentration of elemene for triggering DNA fragmentation was much lower than that for 24-h treatment. When morphological changes in elemene-treated HL-60 cells

Table II: Effect of elemene on the growth of mouse ascite tumors.

Tumor	Dose (mg/kg)	Route	Treatment schedule	Increase of life span (%)
EAC	50	i.p.	d1 - d7*	11
	75	i.p.	d1 - d7	149
	100	i.p.	d1 - d7	187
Sarcoma 180	50	i.p.	d1 - d7	92
	75	i.p.	d1 - d7	122
	100	i.p.	d1 - d7	152

EAC: Ehrlich ascites carcinoma. *Day after tumor implantation.

Table III: Effect of elemene on the growth of subcutaneously implanted solid tumors.

Tumor	Dose (mg/kg)	Route	Treatment schedule	Inhibition of tumor growth (%)
Mouse Lewis lung cancer	60	i.p.	d1 - d7*	17.5
	80	i.p.	d1 - d7	20.3
	100	i.p.	d1 - d7	39.8
Rat Walker 256 sarcoma	60	i.p.	d1 - d7	9.7
	80	i.p.	d1 - d7	28.3
	100	i.p.	d1 - d7	29.5

*Day after tumor implantation.

Table IV: Effect of elemene on cell cycle progression of HL-60 cells.

Concentration (mg/l)	Time (h)[a]	G_1	S	G_2M
0	4	35.7[b]	50.4	13.9
20	4	26.8	59.0	14.2
0	24	36.8	50.8	12.4
20	24	21.8	74.8	3.4
0	48	40.7	46.0	13.3
20	48	47.2	48.9	3.9

[a]Hour after elemene administration. [b]Percent.

were examined by transmission electron microscope, distinct apoptotic morphological features were also observed and consisted of condensed chromosomes and apoptotic bodies. The characteristic ultrastructural alterations in the elemene-treated HL-60 cells were associated with the agent exposure and very similar to those of the cisplatin-treated HL-60 cells. These results indicate that induction of apoptosis contributes to the mechanism of antitumor action of elemene.

In addition, oxygen free radical formation is associated with the generation and development of certain tumors (14). Elemene enhanced serum superoxide dismutase activity in patients with brain tumors (15). It is suggested that the agent has an inhibitory effect on oxygen free radical formation in tumor cells, which may also contribute to its cytotoxic effects.

Indirect immunostimulatory effects

It is known that patients suffering from tumors are immunodeficient and conventional chemotherapeutic regimens further compromise the patients' immune system. However, elemene not only exhibited direct cytotoxic effects on various tumor cells, but also enhanced the depressed immune responses of patients. As shown in Table V, the numbers of peripheral blood T-cell subsets in patients with various tumors were decreased. Elemene increased the percentages of T3 (total T), T4 (th/i) and T8 (ts/c) cells with decreased T4/T8 ratios (16). Elemene administered by intercavernous injection also stimulated LAK activity of effusion-associated lymphocytes (EAL) in malignant pleural effusion. Moreover, elemene increased leukocytes and lymphocytes counts, as well as red cell membrane complement 3 receptors in peripheral blood of patients treated with other chemotherapeutic drugs (Table VI) (11).

Pharmacokinetics

The preliminary studies on absorption, distribution and excretion of elemene have been performed in mice using [^{3}H]-elemene (11). The blood concentration-time curve of the drug was shown to fit a two-compartment open model. Its half-life ($t_{1/2}$) was as follows: $t_{1/2\alpha}$ = 11.2 min, $t_{1/2\beta}$ = 10.5 h. Low absorption after oral administration was observed; the bioavailability was only 18.8%. Peak blood levels were reached at about 6 h after oral administration. After i.v. administration, high concentrations were found in the lungs, spleen, liver and lymph glands, and concentrations were highest in the lungs. Elemene also penetrated the brain through the blood-brain barrier after i.v. or oral administration. In addition, it was distributed into tumor tissues.

In the case of i.v. or oral administration, 26.1% of total dose administered was excreted from urine, 2.2% via feces and 38.5% via bile within 24 h. As a volatile oil, elemene has more distribution in the lungs, so the respiratory system is its main route of excretion. Such pharmacokinetic characteristics should be taken into consideration for the clinical use of this compound.

Toxicity

Acute lethal doses (LD_{50}) of elemene are 270 mg/kg i.v. and more than 5 g/kg i.g. (11). Elemene had slight suppressive effects on the central nervous system, but no effects on cardiovascular and respiratory systems were observed. The hereditary tests also did not show any

Table V: Effect of elemene on peripheral blood T-cell subset count and LAK activity of effusion-associated lymphocytes in malignant pleural effusion in patients with various tumors.

Group	T3 (%)	T4 (%)	T8 (%)	T4/T8	LAK activity (%)
Normal donors	47.8	37.4	24.1	1.51	-
Patients with tumors	37.9	32.4	18.9	1.79	10.5
Elemene	40.9	34.1	21.2	1.55	35.1

Table VI: Effect of elemene on chemotherapy-inhibited immune functions in patients with tumors.

Group	Leukocytes (x 10^9 cells/l)		Leukocytes (x 10^9 cells/l)		C3 receptors	
	Without C	With C	Without C	With C	Without C	With C
Control	6.3	3.9	1.9	1.4	9.2	8.9
Elemene	5.9	5.5	1.9	1.8	9.4	13.6

C: chemotherapy.

Table VII: Therapeutic effect of elemene by intrapleural injection in malignant pleural effusions.

Tumor	Response			Total	CR+PR
	CR	PR	No		
Lung carcinoma	24	70	33	127	94
Breast carcinoma	6	4	3	13	10
Lymphoma	2	2	2	6	4
Ovarian carcinoma	1	3	1	5	4
Others	1	9	2	12	10
Total	34	88	41	163	122

CR: complete remission. PR: partial remission. No: no response.

mutagenic effect of the agent (17). In rats and dogs, continuous intravenous injection of elemene (less than 30 mg/kg) for 90 days caused no marked drug-related changes in body weight, organ weight, tests for hematology, serum chemistry, urinalysis or ECG (11).

In clinical trials of elemene, no myelosuppression was observed. There were also no liver, cardiac or renal toxicities. Fever, local pain, phlebitis and gastrointestinal reactions were the major adverse effects but were well tolerated by most patients (11).

Clinical Studies

Elemene was especially effective in advanced tumors with malignant pleural or peritoneal effusions. In a prospective, multicenter phase II clinical trial (18), elemene was administered at a dose of 200 mg/m^2 by intrapleural injection once every week for 1-2 weeks or 300-400 mg/m^2 by intraperitoneal injection once or twice every week for 2 weeks. Complete remission (CR) and partial remission (PR) rates were 20.9 and 54.0%, respectively, in patients with pleural effusion and total response rate was 74.8% (Table VII). CR and PR rates were 38.1 and 36.4%, respectively, in patients with peritoneal effusion and total response rate was 75.0% (Table VIII).

Phase III clinical trials of elemene have also been conducted to evaluate its efficacy in the management of malignant effusions (19). Four hundred and eighty-four patients, including 313 with pleural effusion and 171 with peritoneal effusion, were evaluable. The response rates were 77.6% in patients with malignant pleural effusion and 66.1% in patients with malignant peritoneal effusion. It was concluded that elemene administered by local injection is an active agent in the management of malignant effusions.

Table VIII: Therapeutic effect of elemene by intraperitoneal injection in malignant peritoneal effusions.

Tumor	Response			Total	CR+PR
	CR	PR	No		
Ovarian carcinoma	27	19	5	51	46
Gastric carcinoma	5	8	5	18	10
Pancreatic carcinoma	0	1	3	4	1
Hepatoma	0	1	2	3	1
Others	0	2	6	8	2
Total	32	31	21	84	63

Table IX: Therapeutic effect of elemene by intravenous administration in advanced lung cancer.

Tumor	Response			Total	CR+PR
	CR	PR	No		
Primary lung cancer	2	14	30	46	16
Secondary lung cancer	0	1	6	7	1
Total	2	15	36	53	17

Table X: Therapeutic effect of elemene by local infiltration in superficial cancers.

Tumor	Response			Total	CR+PR
	CR	PR	No		
Uterine cervix carcinoma	11	5	5	21	16
Lung cancer	2	8	7	17	10
Brain cancer	1	1	3	5	2
Hodgkin's lymphoma	0	1	0	1	1
Gastric carcinoma	0	1	0	1	1
Thyroid carcinoma	0	1	0	1	1
Nasopharyngeal carcinoma	1	0	0	1	1
Esophageal carcinoma	0	1	0	1	1
Total	15	18	15	48	33

Elemene was also effective by hepatic artery injection for primary hepatocarcinoma (PHC) (20). In 71 patients with PHC, 2 CRs and 38 PRs were observed. Elemene administered intravenously has also been recommended for clinical treatment of advanced lung cancer with 32.1% total response rate (Table IX). It was also effective for advanced lung cancer by bronchial artery injection or pulmonary artery injection (21, 22). It is known that elemene penetrates the blood-brain barrier. Therefore, the agent was also used for the treatment of brain cancers by intracarotid artery infusion; a 63.3% response rate (8 CRs and 11 PRs in 30 patients) was reported (11).

Elemene was effective by local infiltration for superficial cancers with a 63.5% total response rate (Table X). Combination drug treatment in tumor therapy is becoming more and more important. When elemene was combined with other chemotherapeutic drugs, radiotherapy or immunotherapy, a synergistic inhibition of tumor growth was found in patients with various tumors. In addition, elemene has also been used for treating other tumors such as dermatocarcinoma, osteosarcoma, multiple myeloma and leukemia, with favorable results reported in some patients (11).

Conclusions

Elemene, a new natural product isolated from Chinese medicinal herb, exhibited broad antitumor

activity. However, the sensitivity of various tumors to the drug may differ. It is also noteworthy that methods of therapeutic use and pharmacokinetics characteristics may influence the efficacy of elemene therapy. Therefore, close attention should be paid to these factors in cancer treatment with the drug.

Conventional tumor chemotherapy has a low degree of specificity, indicating that resulting side effects have limited its efficacy. Therefore, it is important to develop cancer-specific agents with greater selectivity (13). Although elemene does not produce a significantly better chemotherapeutic effect than those drugs already in use, fewer side effects were reported in clinical trials with the compound. Elemene may not rapidly kill proliferating non-malignant cells such as bone marrow stem cells. No overt signs of drug-induced toxicity on liver and kidney function, and no myelosuppression were observed. Moreover, it has been demonstrated that elemene exhibits immunostimulatory activities. These data suggest that elemene has the potential to be developed as a new antitumor drug with high specificity.

Source

Dalian Jin Gang Pharmaceutical Co., Ltd. (P.R. China).

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