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Research Paper

An investigation of the ability of elemene to pass through the blood–brain barrier and its effect on brain carcinomas

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Abstract

Objectives Elemene is a chemical extracted from plants. It has demonstrated anti-tumour capability. Although widely studied, there has been little reported regarding its tissue distribution. Our aim was to rectify this.

Methods The tissue distribution of elemene was studied after intragastric or intravenous administration in rats. The effectiveness of elemene in treating brain tumours was studied using the G-422 tumour cell model in mice.

Key findings Elemene had a higher concentration in the lungs, spleen and livers than other tissues of normal rats after intragastric and intravenous administration, while the concentration in the gastrointestinal tract was greater after intragastric administration. Elemene molecules were also detected in the rats' brain tissue. Elemene had a therapeutic effect on mice inoculated with G-422 cells both intracranially and subcutaneously. The best life-extending rate and the best tumour-inhibiting rate of elemene were 64.43% and 34.46%, respectively, when 80 mg/kg elemene was used for treatment.

Conclusions The results from the tissue distribution study showed that elemene can pass through the blood–brain barrier. The therapeutic experiments showed that elemene is effective in treating cerebral malignancy.

Keywords anti-cancer activity; brain carcinoma; elemene; tissue distribution

Introduction

Elemene is a chemical compound that can be extracted from numerous plants.[1] More that fifty different plants have been found to contain elemene.[2] These plants include *Radix inulae*, *E. wenyujin* C. and others.[3] These plants grow in tropical areas around the world. Elemene has three isomers, β-, δ- and γ-, with β-elemene as the main component (85%) and the other two as minor components.

Preparations made from *Curcuma aromafica* Salisb. that contains elemene have been a part of traditional Chinese medicines for centuries. Such preparations have been used internally and topically for a wide variety of ailments.

Elemene has demonstrated anti-tumour characteristics; numerous reports have appeared in Chinese scientific literature. In 1993, β-elemene was officially approved as an anti-cancer drug in China. After that, injectable emulsions of β-elemene were used for the first-line treatment of pleural malignancies, chest and abdominal ascites, malignant brain tumours and cancers of the respiratory and digestive tracts, and for the second-line treatment of cancers of female reproductive organs, breast cancer, metastatic bone cancer, skin cancer, lymphoma and leukaemia.[4–6] However, little is known regarding the tissue distribution of elemene. To obtain experimental evidence of elemene passing through the blood–brain barrier and to offer necessary information to contribute to its clinical applications, we carried out a tissue distribution study of elemene using rats as a model, and also did a study on the therapeutic effects of elemene using tumour-inoculated mice. In this article, we report the preliminary results of the tissue distribution study and the therapeutic effect of elemene on tumours.

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Materials and Methods

Elemene tissue distribution

Clinically used elemene oral emulsion (0.2 g/20 ml) and elemene injectable emulsion (25 mg/5 ml) were supplied by Dalian Holley Kingkong Pharmaceutical Co. Ltd (Dalian, China). Sprague–Dawley rats (clean grade, 208 ± 24 g) were purchased from the animal center of the Zhejiang Academy of Medical Sciences. The rats were fasted with water freely available for 24 h before the experiments. The experiments were separated into two groups. The rats in the first group were given elemene emulsion intragastrically at a dose of 200 mg/kg. The rats in the second group were given the drug intravenously at a dose of 100 mg/kg. For both groups, a single dose was used for each rat. Five parallel replicates were conducted for each time point in each group. In the first group, after drug administration for a defined period of 20, 120, 480 and 1440 min, the rats were sacrificed by the decollation method and tissues from various organs were cut. The cut tissue was mixed with saline in a ratio of 1 : 2. The mixture was homogenized and then centrifuged to obtain the supernatant. The supernatants were analysed using liquid chromatography. The chromatographic column was HC-C18 (4.6 mm × 150 mm, 5 μm; Agilent Technologies Inc., Santa Clara, USA). The mobile phase was methyl cyanide–water (80 : 20 v/v). The flow rate was 1 ml/min. The operating temperature was 25°C. The detection wavelength was 210 nm.

Effect of elemene on tumours

Clinically used elemene injectable emulsion was the same as that used in the tissue distribution study. Naked mice, aged six weeks, were obtained from the Shanghai Cancer Institute. Elemene at a dose of 80, 40 and 20 mg/kg per day was injected intravenously into tumour-bearing mice for ten days. Water solution with emulsifiers used in making the elemene emulsion was used as a placebo. The placebo solution was injected intravenously into the control group. Two groups of tumour-bearing mice were used.

The first group was ten mice intracranially inoculated with 0.05 ml (2×10^7 cells/ml) cell suspension of tumour G-422. The second day after inoculation, elemene was intravenously injected into five mice daily for 10 days and placebo solution was intravenously injected into the other five mice daily for ten days as controls. The mice were observed for 30 days to determine their survival time and the life-extending rate (Equation 1) compared with those of the placebo-treated tumour-bearing mice.

$$\text{Life-extending rate \%} = 100\times(\text{average survival days of the drug-treated group} - \text{average survival days of the placebo-treated group})/\text{average survival days of the placebo-treated group} \quad (1)$$

The second group was ten mice subcutaneously inoculated with 0.2 ml (1×10^7 cells/ml) cell suspension of tumour G-422 into the armpit of the mice. The second day after inoculation, elemene was administered intravenously to five mice on a daily basis for ten days and placebo solution was intravenously injected into the other five mice also on a daily basis for ten days as controls. After observing for three weeks, the mice were killed by decollation and tumours were removed from the body. The weight of the tumour was used to calculated the tumour-inhibiting rate as follows:

$$\text{Tumour-inhibiting rate \%} = 100\times(\text{weight of non-treated tumour} - \text{weight of treated tumour})/\text{weight of non-treated tumour} \quad (2)$$

Elemene was given to the tumour cell-inoculated mice only one day after the tumour cell inoculation. We did not wait until the tumours were established in the mice because elemene is not a tumour cell-killing chemical; rather, it can inhibit the tumour cell growth in the animal body. If the elemene were given to the mice in whom the tumour had been established, the drug effectiveness on the tumour would be poorer.

Statistical methods

Statistical analysis of the therapeutic effect study was carried out using a one-way analysis of variance. In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's Honestly Signficant Difference test. $P < 0.05$ or $P < 0.01$ denoted significance in all cases.

Results

The results of the study of elemene tissue distribution using the rat model are shown in Tables 1 and 2. From these tables, one can find the following facts. The concentration of elemene was reduced as time passed by. Elemene reached a higher concentration in the lungs, spleen and liver than in other tissues in normal rats after intragastric and intravenous administration, while the gastrointestinal tract had the highest concentration of drug after intragastric administration. One can also find that elemene molecules were detected in the rats' brain tissue. Thus, one may reach the conclusion that elemene can pass through the blood–brain barrier.

The research to test the effectiveness of elemene in treating brain tumours was conducted using G-422 tumour cells in mice as the model. The results are shown in Tables 3 and 4. The life-extending rate and the tumour-inhibiting rate

Table 1 Distribution of elemene after intragastric administration to normal rats

Sample	Concentration (μg/ml)			
	20 min	120 min	480 min	1440 min
Brain	4.52 ± 0.05	5.98 ± 0.12	5.81 ± 0.10	3.01 ± 0.10
Lung	23.70 ± 1.50	7.56 ± 0.23	6.18 ± 0.28	3.69 ± 0.25
Spleen	21.03 ± 0.57	12.51 ± 0.20	9.05 ± 0.22	5.20 ± 0.25
Kidney	14.69 ± 0.10	8.70 ± 0.39	8.65 ± 0.18	4.16 ± 0.36
Heart	7.20 ± 0.13	14.15 ± 1.89	7.93 ± 0.35	4.18 ± 0.17
Stomach	94.50 ± 18.18	212.57 ± 16.37	93.63 ± 12.05	25.44 ± 3.01
Intestine	53.51 ± 5.15	36.74 ± 1.13	19.53 ± 0.79	9.63 ± 0.12
Liver	16.79 ± 0.61	12.99 ± 1.02	12.17 ± 0.43	5.09 ± 0.06

Five rats were used for each time point of the study.

Table 2 Distribution of elemene after intravenous administration to normal rats

Sample	Concentration (μg/ml)		
	20 min	120 min	480 min
Brain	14.42 ± 0.47	5.37 ± 0.43	2.24 ± 0.22
Lung	9.46 ± 0.39	7.60 ± 0.57	4.51 ± 0.37
Spleen	11.57 ± 0.32	9.18 ± 0.41	4.04 ± 0.10
Kidney	24.18 ± 5.39	6.71 ± 0.18	2.34 ± 0.17
Heart	12.17 ± 0.99	7.19 ± 0.13	3.15 ± 0.17
Stomach	13.97 ± 1.17	11.39 ± 0.52	4.09 ± 0.23
Intestine	14.30 ± 1.24	8.76 ± 0.21	3.33 ± 0.11
Liver	31.12 ± 2.97	9.99 ± 0.47	3.43 ± 0.22

Five rats were used for each time point of the study.

Table 3 Effect of elemene injectable emulsion in treating mice intracranially inoculated with G-422 tumour cells

Dose (mg/kg)	Average survival days	Life-extending rate (%)
80	$15.7 \pm 1.4^{***}$	64.43 ± 3.57
40	$14.8 \pm 1.4^{***}$	54.20 ± 2.21
20	$14.2 \pm 1.5^{***}$	48.23 ± 1.04

The average survival days of the control group was 9.6 ± 1.5 days. Data are means ± SD, $n = 5$. $^{***}P < 0.01$ compared with control group.

Table 4 Effect of elemene injectable emulsion in treating mice subcutaneously inoculated with G-422 tumour cells

Dose (mg/kg)	Average tumour weight (g)	Tumour-inhibition rate (%)
80	$1.47 \pm 0.06^{***}$	34.46 ± 1.11
40	$1.54 \pm 0.05^{***}$	30.71 ± 0.86
20	$1.68 \pm 0.06^{**}$	24.55 ± 1.41

The average weight of tumour for the control group was 2.22 ± 0.12 g. Data are means ± SD, $n = 5$. $^{***}P < 0.01$, $^{**}P < 0.05$, compared with control group.

in mice were used to express the effectiveness of the elemene against G-422 tumour cells. From Tables 3 and 4, one can see that elemene had a therapeutic effect in mice inoculated with G-422 cells both intracranially and subcutaneously. This tells us that the elemene is capable of passing through the blood–brain barrier. So, one can reach the conclusion that elemene should be a good drug in treating cerebral malignancy. Tables 3 and 4 also show that the higher dose had a better effect in treating the tumour. The best life-extending rate of the mice and the best tumour-inhibiting rate of elemene were 64.43% and 34.46%, respectively, when 80 mg/kg elemene was used for treatment.

Discussion

Cerebral glioma and cerebral metastatic carcinoma are the most common carcinomas of brain and among the deadliest human cancers. They are aggressive, highly invasive and neurologically destructive tumours, which respond poorly to chemotherapy.[7–10] The current treatments for brain tumours are surgery, radiation therapy, chemotherapy or biological therapy. These treatment methods are painful and produce side effects. There is an urgent need for effective brain-tumour-treating drugs to prolong patients' life and to improve their life quality. Since the brain poses a large problem for drug delivery, chemotherapy is usually co-delivered with a blood–brain barrier permeation enhancer (e.g. mannitol).

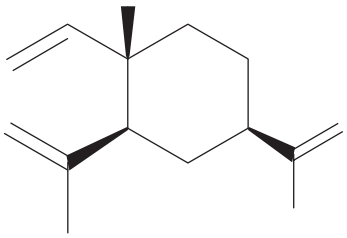

Figure 1 The chemical structure of β-elemene ($C_{15}H_{24}$, MW: 204.35)

From the chemical structure of elemene (Figure 1), one can observe that the elemental components of elemene are carbon and hydrogen only. The molecular weight of elemene is very small. All of the virtues above make elemene different from other anti-tumour organic drugs such as paclitaxel, camptothecin, vinblastine, etc.

In comparison with other anti-tumour drugs, its lipophilic nature and small molecular weight give elemene a unique character, which includes its ability of passing through the blood–brain barrier, so elemene not only has the ability to manage carcinomas of the lung, liver, stomach, breast, etc., but also has beneficial effects on carcinomas of the brain.[4–6] Therefore, the advantage of elemene in clinical applications may give this new chemical the primary position in treating brain cancers.

Conclusions

Elemene has demonstrated anti-tumour characteristics. Many reports may be found in Chinese scientific literature. However, there is little study regarding the tissue distribution of elemene. To obtain experimental evidence of the characteristic of elemene of passing through the blood–brain barrier and also to offer necessary information for clinical applications, a tissue distribution study of elemene using rats as a model was conducted. A study on the therapeutic effects of elemene using tumour-inoculated mice was also performed. The results from the tissue distribution study show that elemene can pass through the blood–brain barrier. The therapeutic experiments showed that elemene has an effect in treating cerebral malignancy.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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