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Original article

Evaluation of *in vivo* antitumor activity of cleistanthin B in Swiss albino mice

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ABSTRACT

To evaluate the *in vivo* antitumor activity of cleistanthin B in Ehrlich's ascites carcinoma (EAC) and Dalton's ascites lymphoma (DAL) cell lines induced malignant ascites mouse models and DAL cell line induced solid tumor mouse model. All animals were injected with 2×10^6 EAC/DAL cells i.p./s.c. to induce malignant ascites and solid tumor and treated with 5-fluorouracil (5-FU) 20 mg/kg or cleistanthin B for 10 days. Cleistanthin B was given at three doses *viz.* 25, 50 and 100 mg/kg. The percentage increase in life span and the overall survival in malignant ascites animals and the tumor volume in solid tumor animals were measured. The haematological parameters were assessed in all animals before and 2 weeks after the treatment. Cleistanthin B 50 mg/kg and 5-FU significantly prolonged the life span (>25%) of malignant ascites tumor bearing animals. The overall survival was significantly improved by both. Only cleistanthin B 50 mg/kg significantly reduced the elevated WBC counts in EAC tumor bearing animals. Both 5-FU and cleistanthin B 50 mg/kg reversed the malignancy induced increase in neutrophils and platelet counts and decrease in lymphocyte counts but not to the normal range. Only 5-FU significantly reduced the solid tumor volume. None of the three doses of cleistanthin B was effective against the solid tumor. Cleistanthin B has antitumor activity against EAC and DAL tumor mice but it is not as effective as 5-FU. At 50 mg/kg dose cleistanthin B exerts significant antitumor activity compared to 25 and 100 mg/kg dose. Its effect on WBC count is higher and advantageous when compared to 5-FU. But cleistanthin B in the doses used is not effective against solid tumor.

1. Introduction

Cancer is one of the leading causes of death worldwide and the burden is increasing day by day. Though we have a number of anticancer agents now, adequate control of cancer is still lacking. Hence there is a persistent demand to develop newer and more effective anticancer drugs which can help tackle this problem. The major groups of anticancer drugs such as vinca alkaloids, taxanes, camptothecins and epipodophyllotoxins which are currently a part of many standard anticancer regimens are derived from plants.[1] Cleistanthin B is an arylnaphthalene lignan lactones glycosides, the major phytoconstituents responsible for most of the toxicological and pharmacological actions of *Cleistanthus collinus*, a plant commonly found in the states of southern India.[2] Apart from cleistanthin B, plant has cleistanthin A, collinusin, and diphyllin.[3]

Cleistanthin B also present in *Dysosma versipellis* (Berberidaceae), a Chinese herb widely used to clear sputum, kill parasites and treat epidemic encephalitis B and epidemic parotitis,[4] and *Hypoestes purpurea* (Acanthaceae), a tropical herb eaten as spinach, used as a poultice for sore eyes.[5,6] Cleistanthins A and B were reported to possess hypotensive[7] and diuretic[8] properties. While these compounds have no activity on motor function,[9] they were demonstrated to exhibit significant antitumor activity.[10–14] Most of the studies about antitumor activity of these compounds are based on *in vitro* assays. The GI_{50} (50% growth inhibition) values are lower

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for tumor cells compared to normal cells.[11,13] They exert cytotoxic activity on various cancer cell lines by interfering with cell cycle progression, causing DNA damage and inducing apoptosis.[10] While cleistanthin A has *in vivo* antitumor activity against Dalton's ascites lymphoma (DAL) and S-180 sarcoma tumor bearing animals by prolonging the life span and reducing the tumor volume,[11] there is no report of the *in vivo* antitumor activity of cleistanthin B so far. Hence the present study was focused to evaluate the *in vivo* antitumor activity of cleistanthin B in Ehrlich's ascites carcinoma (EAC) and DAL cell line induced malignant ascites and solid tumor in mice.

2. Materials and methods

2.1. Test compound

Cleistanthin B was isolated from the leaves of *C. collinus* (Euphorbiaceae) as described in our previous study.[15] Taxonomically identified plant leaves were collected in the regions of Pondicherry district and it was certified by Botanical survey of India, Coimbatore (BSI/SC/5/23/08-09/Tech.241).

2.2. Cancer cell lines

EAC and DAL cell lines (Amala Cancer Research Center, Thrissur, Kerala) were maintained by weekly intraperitoneal transplantation of respective tumor cells (2×10^6 cells per mouse) in stock animals.

2.3. Chemicals

Carboxymethyl cellulose sodium (CMC) (Lobachemie, Mumbai), phosphate buffered saline (PBS) (Media labs, Mumbai), Diethyl ether (Thermo Fisher Scientific India Pvt Ltd, Mumbai) and 5-fluorouracil (5-FU) (Ceon labs, Andhra Pradesh) were used for the study.

2.4. Instruments

Automated haematology analyzer (SYSMEX XS-1000i; Sysmex Corporation, Kobe, Japan) was used for the complete blood cell counts.

2.5. Animals

Female Swiss albino mice were obtained from the central animal house of JIPMER, Pondicherry and they were maintained under standard laboratory conditions throughout the study. The animals were fed with standard rodent pellet feed (Amrut feeds, Sangli, India) and water *ad libitum.* Adult mice weighing 20–30 g were used for the experiments. The study protocol was approved by the Institutional Animal Ethics Committee (No: Jip/Micro/Jiaec/2012) and all the animal experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

2.6. Study design

An exploratory study was designed to evaluate the *in vivo* antitumor activity of cleistanthin B using different mouse tumor models. Study I and II were carried out with EAC and DAL cell lines induced malignant ascites mouse models, respectively. DAL cell line induced solid tumor mouse model was used for study III. Three doses of cleistanthin B *viz.* 25, 50 and 100 mg/kg were chosen based on the results of a toxicity study done previously.[16] The animals were divided into five different groups as follows:

Group I: Tumor control
Group II: 5-FU 20 mg/kg
Group III: Cleistanthin B 25 mg/kg
Group IV: Cleistanthin B 50 mg/kg
Group V: Cleistanthin B 100 mg/kg

Study I and II used 8 mice in each group while study III used 6 mice per group. Group I served as a positive (tumor) control (0.5% CMC orally) and group II as a comparator control (5-fluorouracil 20 mg/kg; i.p.). Groups III, IV and V were given cleistanthin B orally at the doses of 25, 50 and 100 mg/kg respectively. The study design is summarized in Fig. 1.

2.7. Experimental procedure

On day 1, blood collection from retro-orbital plexus was carried out and the samples (0.3 ml) in EDTA were used for the assessment of haematological parameters such as haemoglobin (Hb) content, red blood cell (RBC) count, total white blood cell (WBC) count, neutrophils (%), lymphocytes (%) and platelet count. On day 2, tumor fluid was withdrawn from the stock animals for EAC and DAL cell lines and the tumor cell count was done using Neubauer chamber under the light microscope. The PBS was added to make a concentration of 1×10^6 cells in 0.1 ml. For tumor induction in study I and II, each experimental animal was injected with 2×10^6 DAL/EAC cells *i.e.* 0.2 ml intraperitoneally. In study III, the solid tumor was induced by injecting *s.c.* injection of 2×10^6 DAL cells in 0.1 ml PBS on right hind limb of each experimental animal. After 24 h of the tumor cells inoculation, the animals were treated according to their respective groups once daily for next 10 days. On day 15, the retro-orbital blood collection was done again for haematological assessment, if the animal was alive.[17] The animals were followed till death or upto 35 days. The parameters for antitumor activity in study I and II were recorded as follows:

Determination of the percentage increase in life span (PILS): It is calculated from the mean survival time (MST) values.[18] The MST for each group was calculated as,

$$\text{MST (days)} = \frac{\text{Total number of days survived by all animals in the group}}{\text{Number of animals in the group}}$$

For each group,

$$\text{PILS (\%)} = [(\text{MST of treated group}/\text{MST of control group}) - 1] \times 100$$

Determination of the overall survival: It was based on the results of survival analysis. In study – III, the antitumor activity was assessed by the reduction of solid tumor volume.

Determination of the solid tumor volume: The solid tumor volume was measured on the seventh day after tumor inoculation and then it was repeated every fifth day until the end of the study. The tumor volume was calculated using the following formula[19]

$$\text{Tumor volume (ml)} = 4/3\pi r_1^2 r_2 \left(\text{cm}^3\right)$$

where, r_1 and r_2 are two perpendicular radii of the solid tumor measured using a vernier caliper.

The haematological parameters of all surviving animals such as haemoglobin, RBC, WBC, neutrophils, lymphocytes and platelets were assessed for all the three studies. A group of six normal mice was studied for assessing their haematological parameters. These normal (control) values were used for comparisons. The tumor bearing animals alive at the end of the study were sacrificed by cervical dislocation.

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