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SHORT COMMUNICATION

REDUCED TOXICITY AND ENHANCED ANTITUMOR EFFICACY OF BETACYCLODEXTRIN PLUMBAGIN INCLUSION COMPLEX IN MICE BEARING EHRICH ASCITES CARCINOMA

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Abstract : Inclusion complex of plumbagin was prepared with betacyclodextrin employing neutralization method. The toxicity of the drug was reduced and the antitumor efficacy was enhanced on complexation with betacyclodextrin.

Key words : plumbagin, inclusion complex, betacyclodextrin, LD_{50}, antitumor, efficacy

INTRODUCTION

Plumbagin (2–methly, 5–hydroxy, 1:4 naphthoquinone) present in *Plumbago zeylanica* and *Plumbago rosea* is prescribed for cancer treatment in the siddha system of medicine (1). It is also reported to act against chemically induced fibrosarcoma in mice and against P388 leukemia *in vitro* (2). It is a highly toxic and acts like a spindle poison by inhibiting cell mitosis at low concentrations. At higher concentrations it exhibits radiomimetic, nucleotoxic (arrest of cell proliferation and decrease in mitotic index, with evidence of chromosomal abberations) and cytotoxic effects (nuclear and cytoplasmic vacuolization, disintegration of cytoplasm, karyopyknosis and nuclear polymorphism (3). Vinca alkaloids bind with protein tubules and block mitosis with metaphase arrest leading to arrest of cell division. The inability to segregate chromosomes during mitosis leads to cellular death (4). A similar mechanism of action may be attributed to plumbagin in inhibiting tumor cell growth.

Both hydrophobic and hydrophilic drugs from inclusion complexes with cyclodextrins. As a result of interactions between the drug and the carrier, the aqueous solubility, stability, dissolution, diffusion and the bioavailability of the drug is increased (5,6,7,8) and irritability is reduced (9). Complexes can be formed by different methods viz kneading, spray drying, neutralisation and freeze drying. The formation of a true complex can be confirmed by ultraviolet spectroscopy, flourescence spectroscopy, infra red, x-ray diffraction, nuclear magnetic resonance, differential scanning calorimetery and molecular modelling (10-13).

Anti-cancer drugs containing quinone nucleus like doxorubicin and daunorubicin at higher concentration, have been shown to induce oxidative stress and cardiac toxicity leading to death (14). Plumbagin, being a quinone derivative may also have similar effects. Side effects and toxic effects include diarrhea skin rashes, increase in WBC and neutrophil count, increase in serum alkaline

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phosphatase levels, acid phosphatase levels, hepatic toxicty etc (15, 16). It has a narrow margin of safety and wide variations in LD_{50} have been reported (15, 17). The present work was undertaken to explore the feasibility of the carrier in reducing the toxicity and enhancing the antitumor efficacy of PLB by forming inclusion complex with BCD.

METHODS

Preparation of neutralisation complex:

The neutralisation complex was prepared by the method of Erden and Celebi 1988 (18). Plumbagin (Sigma Chemicals, USA) and Betacyclodextrin (Nippo Shokuhin Kaako Co., Japan) (1:1 molar ratio) were dissolved in 0.1N sodium hydroxide and 0.1N hydrochloric acid was added dropwise till the complex precipitated, at pH 7.4 to 8.0. The complex was filtered and dried.

Acute toxicity studies and antitumor efficacy:

For tumor and toxicity studies, six to eight weeks old BALB/c mice from an inbred colony, weighing 20-25 g were used. They were maintained in our animal house under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and light and dark cycle (10 and 14 hours respectively). The animals were given sterile food prepared in the laboratory as per the standard formulation (wheat 70%, bengal gram 20%, fish meal 5%, yeast power 4%, sesame oil 0.75% and shark liver oil 0.25%) and water *ad libitum*. Throughout the experiment 5-6 animals were housed in polypropylene cage containing sterile paddy husk as bedding material.

Ehrlich ascites tumor, first obtained from Amala Cancer Centre, Thrissur, Kerala was maintained and propagated by serial transplantation, intraperitoneally, in adult female BALB/c mice. Experimental animals were prepared by injecting 10^6 cells into the intraperitoneal cavity of the mice. The first group (control group) was administered orally, phosphate buffered saline pH 7.4, the second group plain PLB and the third group, complex (containing same amount of PLB) in graded doses 24 hours after tumor innoculation. All the mice were weighed on the day of tumor innoculation and at weekly intervals. Animal survival was monitored upto 40 days and the % increase in life span (%ILS) was calculated by the formula:

$$\%ILS = \frac{(\text{MST of treated group} - \text{MST of the control group})}{\text{MSR of the control group}} \times 100$$

where MST = Mean survival time

Acute oral toxicity:

The dose response survival studies were performed after oral administration of the plain drug and the complex (containing same amount of PLB) in phosphate buffered saline (pH 7.4) in graded doses. Mortality was monitored for a period of 14 days. The LD_{50} values were calculated by the method of Miller and Tainter (19).

RESULTS

The LD_{50} for the plain PLB and the neutralisation complex was found to be 8.51 and 12.88 mg/kg body weight respectively (Table I). At doses of 2, 4 and 6 mg/kg, no mortality was observed for both the complex and the plain drug. However when the dose was increased the mortality was higher in case of plain PLB compared to the complex. At higher doses of plain PLB, toxic symptoms

like diarrhea, ruffling of hair, drownsiness, lethargy were observed. The animals which could survive the lethal effects upto three to four days were able to survive and continue even upto 14 days.

Table II shows the % ILS of tumor bearing mice. The results are clearly indicative of the fact that at equivalent doses the % ILS was significantly higher for the complex compared to the plain PLB.

TABLE I: Acute oral toxicity (14 days) of plain PLB and PLB + BCD complex in BALB/c mice.

Group	*Dose of PLB mg/kg*	*Mortality%*		*Corrected %*		*Probit value*	
		PLB	*PLB+BCD*	*PLB*	*PLB+BCD*	*PLB*	*PLB+BCD*
I	2	0	0	2.50	2.50	3.04	3.04
II	4	0	0	2.50	2.50	3.04	3.04
III	6	0	0	2.50	2.50	3.04	3.04
IV	8	30	10	30.0	10.0	4.48	3.72
V	10	60	30	60.0	30.0	5.25	4.48
VI	12	100	60	97.50	60.0	6.96	5.25
VII	14	–	100.0	–	97.50	–	6.12

n = number of animals (10 in each group)

Corrected percentage :

For the 0% Mortality = 100 (0.25/n)

For the 100 % Mortality = 100 (n - 0.25/n)

LD_{50} of the plain PLB : 8.51 mg//kg body weight

LD_{50} of Neutralisation complex : 12.88 mg/kg body weight

TABLE II : Effect of PLB and PLB + BCD complex in mice bearing Ehrlich ascites cells.

Group	*Treatment*	*Dose mg/kg*	*MST days ± SE*	*% Increase in life span*	*Av wt change on day 14 (%) ± SE*
I	Control	—	19.0 ± 0.08	—	+5.8 ± 0.23
II	Plain PLB	2	21.8 ± 0.15	14.74	+1.3 ± 0.14
III		4	24.0 ± 0.20	26.3	–4.2 ± .11
IV		6	26.2 ± 0.13	37.89	–7.0 ± 0.25
V		8	28.3 ± 0.13	48.95	–9.0 ± 0.29
	PLB + BCD				
VI	complex	2	22.3 ± 0.17	17.37	–2.5 ± 0.41
VII		4	25.4 ± 0.14	33.60	–4.7 ± 0.32
VIII		6	28.0 ± 0.16	47.37	–8.0 ± 0.51
IX		8	30.0 ± 0.09	57.89	–9.0 ± 0.33

t test - All groups significantly different from the control group ($P < 0.001$).

ANOVA - Non significant difference between groups 5&8, 2&6 ($P < 0.05$).

Significant difference between group 4&7 ($P < 0.05$)

Rest all groups significantly different from each other ($P < 0.001$).

DISCUSSION

The reduced irritability and increased safety of drugs like benzodiazepines (20) and Pilocarpine prodrugs (9) by complexation with BCD have been reported. The complexation phenomenon increases the solubility, dissolution and absorption of the drug from the GIT which results in faster absorption of the drug in the systemic circulation. In case of free PLB which is practically insoluble, in our study, may have been absorbed to a lesser extent in the systemic circulation and development of diarrhea at higher doses may be an indication of this fact. Furthermore, plain PLB toxicity may be due to accumulation of the drug or it's metabolites in the tissues for a longer time. Metabolic studies have shown that PLB was not detected in blood up to 24 hours when administered to rats (21). The complex may be eliminated from the body after getting metabolized at a faster rate. The LD_{50} values of alcoholic extract of PLB was found to be 2 mg/kg and 10 mg/kg body weight by i.p. and oral route respectively (15). The LD_{50} of niosomal encapsulated PLB and plain PLB was found to be 9.34 and 7.99 mg/kg body weight by i.v. route (16). The enchanced antitumor efficacy of complex at similar doses compared to plain PLB again indicates that complexation increases the solubility of PLB so that it gets absorbed faster in systemic circulation to give a better antitumor activity. Faster absorption and better bioavailability for cyclodextrin complexes have been reported (22, 23).

Uma Devi et al (24) have reported the antitumor efficacy of alcoholic extract of PLB (50 mg/kg body weight + radiation) in mice bearing Ehrlich ascites cells and found that the ILS was 57.89 percent. Bimodality (Drug + Radiation) and the single modality (Drug) treatment in our studies yielded almost the same results.

The above mentioned reasons may explain the reduced toxicity and enhanced antitumor efficacy of the complex compared to plain PLB.

Thus a preliminary study of PLB complexation with BCD resulted in reduced toxicity and better antitumor activity. The exact pharmacokinetics of PLB in terms of systemic absorption may have to be further confirmed by bioavailability studies, tissue distribution and mechanism of metabolism using radiolabelled plumbagin.

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