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ANTITUMOUR EFFECT OF ABRIN ON TRANSPLANTED TUMOURS IN MICE

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Abstract : Abrin, a galactose specific lectin was purified using sepharose 4B affinity column from seeds of *Abrus precatorius*. It exhibited antitumour activity in mice when used at a sublethal dose of 7.5 µg/kg every alternate day for 10 days. Both intralesional and intraperiloneal (ip) administration of abrin was effective in reducing solid tumour mass development induced by Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells. DLA cell line was more sensitive to abrin than EAC. Abrin when injected ip increased the life span of ascites tumour bearing mice. Abrin when used simultaneously with tumour cells brought about maximum antitumour effect. On developed tumour masses, abrin administration brought about significant reduction in tumour volume, especially in DLA induced tumours. Prophylactic administration of abrin was found ineffective.

Key words : *Abrus precatorius* abrin lectins antitumour mice

INTRODUCTION

Abrus precatorius (Rosary Pea/Jequiriti bean) is classified under the family Leguminoseae and subfamily Papilionoideae. It is a climber plant, abundantly seen in rainfed tropical countries and the seeds of which are deadly poisonous if taken orally.

A saccharide binding, highly toxic, lectin glycoprotein called abrin has been isolated from *Abrus* seeds (1). Abrin has got greater specificity for galactose and galactose containing structures (2) and can agglutinate rabbit or sheep erythrocytes (3).

Several methods for isolating the abrin from Abrus seeds have been reported, including using single step affinity chromatography (4). The purified Abrus toxin is composed of two polypeptide chains, one of which is responsible for its cytotoxic effect and the other as a carrier moiety for binding the toxic chain to galactose containing structures present on the surface of cells (5, 6). The toxic subunit A is attached to the agglutinin subunit B by means of a disulfide bond (7).

Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and

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isoelectric focussing techniques, three toxins and two agglutinins have been identified and characterised from the abrin namely abrin I, II and III (toxins) and APA-I and II (agglutinins) (8, 9).

Subunit A has been widely used for the construction of immunotoxins (10) and subunit B has been used as a mitogen (5). Toxicity and cytoagglutinating studies with purified abrin on various *in vitro* cell cultures, both normal and transformed, have been reported (11, 12, 13), but little is known about *in vivo* tumour reducing property of abrin. Hence this study was undertaken to find out the antitumour activity of abrin at sublethal concentration on different tumour models in mice.

METHODS

Purification of abrin

Seeds of red variety of *Abrus precatorius* were collected from Central Kerala districts during April 2000 and authenticated by the botanist at the centre. Ten grams of seeds were crushed, powdered and homogenized at 4°C in 50 ml PBS containing 5% NaCl. This suspension was centrifuged at 20,000 RPM for 30 minutes at 4°C. The supernatant was passed through a Sepharose 4B affinity column (1.5 × 15 cms), which was earlier equilibrated with PBS and the flow rate was adjusted to 0.2 ml per minute. Column was washed extensively with PBS until the eluate showed less than 0.01 UV absorbance at 254 nm. Finally affinity elution using 2 bed volumes of eluant D-galactose (0.1 M) was done and the fractions of 1.2 ml each were collected. Those fractions showing absorbance peak of more than 1.20 were pooled (Fig. 1). The pooled samples was extensively dialyzed with PBS overnight and then stored at –20°C (Stock solution).

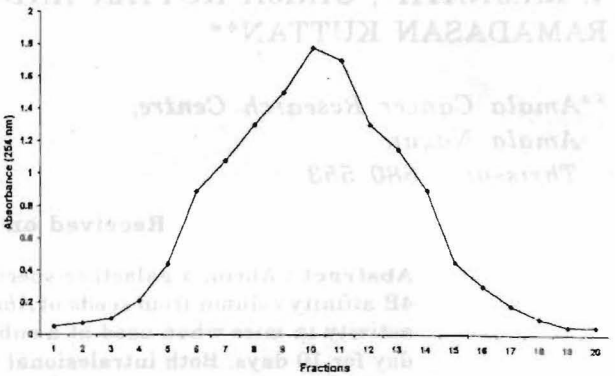

Fig. 1: OD of galactose eluted samples.

Protein content

The protein content of crude extract, supernatant, flowthrough fractions and galactose eluted pooled samples was estimated by Lowry's method (14) and the yield of purified protein (%) was calculated.

Haemagglutintion and Inhibition (HA and HI) assays

HA assay was carried out in disposable plastic 96 well microtiter plates using trypsinized sheep red blood cells (SRBCs) as antigen. 100 µl of the test sample (supernatant and purified samples) was serially diluted with PBS and an equal volume of trypsinised SRBC (1%) was added and incubated for 3 hours at room temperature. The degree of agglutination was evaluated macroscopically and the titer was calculated as the reciprocal of highest dilution of sample which showed visible agglutination.

HI assay was performed by setting up a series of wells containing twice the minimum haemagglutination dose of abrin (50 µl) together with different concentration of D-galactose (50 µl). After incubating for 1 hour at room temperature, 50 µl of 2% trypsinized SRBC suspension was added to each well and allowed to stand for 3 hours. The degree of hemagglutination was examined and the maximum dilution of D-galactose showing hemagglutination inhibition was recorded.

Determination of toxicity of the abrin preparation

Swiss albino mice (females, 25 g, 6–7 weeks old) purchased from National Institute of Nutrition Hyderabad and Balb/c mice (males, 30 g, 8 weeks old) purchased from Veterinary College, Thrissur, Kerala were used for the trials. They were kept in well ventilated cages and were given mice chew and water *ad libitum*. Eight groups of mice (8 animals/group) were employed for determining LD_{50} dose of abrin. Stock solution of abrin was diluted with PBS and eight different concentrations of purified abrin having doses of 25 ng, 75 ng, 150 ng, 300 ng, 600 ng, 1500 ng, 3000 ng and 6000 ng were injected on every alternate day intraperitoneally to group I to VIII for a total duration of 10 days (5 doses) in all.

To find out the safest dose of abrin which could be used for further experiments, another trial was conducted using three groups of Balb/c mice (8 animals/group) at lower concentrations than LD_{50} dose (1/2, $1/10^{th}$ and $1/2^{th}$ of LD_{50} dose). Abrin administration was carried out intraperitoneally on every alternate day for a period of 10 days (5 doses). Body weight changes were noted and blood was collected from caudal vein on every third day for a period of 18 days. The hematological parameters such as hemoglobin content and RBC count were recorded and the values were compared with pretreatment values.

Determination of the effect of abrin on ascites tumours

Dalton's lymphoma ascites (DLA) cells (originally grown from a spontaneously grown tumour of mouse thymus) and Ehrlich's ascites carcinoma (EAC) cells were propagated in Swiss Albino mice by injecting 1×10^6 cells intraperitoneally.

On the day of experiment, DLA cells and EAC cells were aspirated separately, washed with PBS and a cell suspension made, containing 1×10^6 cells in 0.1 ml of PBS. Animals were divided into four groups (6 mice/group). Group I and II were inoculated with DLA cells ip whereas group III and IV with EAC cells ip. Animals in Group I and III were untreated whereas those in Group II and IV received abrin at the rate of 7.5 µg/kg body weight, ip on every alternate day from 11^{th} day of tumour induction for a period of 10 days. The death pattern of animals due to tumour burden was noted for 120 days and the percentage increase in life span (ILS) was calculated using the formula % ILS = T-C/C x 100 where T and C represent the number of days that treated and control animals survived respectively.

Determination of the effect of abrin on solid tumours

Using DLA and EAC cells, solid tumors were induced in mice and the effect of abrin was tested on them. Abrin was administered

in three different modalities namely simultaneously with tumour cells, after tumour development and prophylactically.

Simultaneous administration of abrin

Mice were divided into 6 groups (8 Balb/c mice/group). Group I, II and III received DLA cells and group IV, V and VI received EAC cells. Respective tumour cells (1 x 10^6 cells/0.1 ml PBS) were injected into the right hind limb of all the animals intramuscularly (im). Mice of Group I and IV were kept as untreated controls. On the same day Group II and V received abrin (0.1 ml) @ 75 µg/kg intralesionally into the same site where tumour cells were previously inoculated. Group III and VI received the abrin dose by intraperitoneal route. Administration of abrin was continued for 5 alternate days. The measurement of tumour mass radii were taken from 11th day of tumour induction and was repeated on every 5th day for a period of 30 days. The volume of tumour mass was calculated from the formula V in ml or cc = 4/3 π r^3 where r is the mean of r_1 and r_2 which are two independent radii of the tumour mass.

Administration of abrin after tumour development

Six groups of animals were employed as mentioned above for the experiment. Tumour cells (1 x 10^6 cells) were injected in on the right hind limb. Mice of Group I and IV were kept as untreated controls. Abrin @ 7.5 µg/kg (0.1 ml) was injected intralesionally to Group II and V animals on the 11th day of tumour induction. Group III and VI received abrin dose (75 µg/kg) by ip route. Administration of abrin was continued for 5 alternate days. The measurement of tumour mass radii was done on every 5th day for a period of 30 days. The volume of tumour mass was calculated from the formula given above.

Prophylactic administration of abrin

Four groups of Balb/c mice (8 animals/group) were employed for this trial. Mice of Group I and III were kept as untreated controls. 5 doses of abrin (7.5 µg/kg) were given ip on alternate days for a total period of 10 days to Group II and IV. On the 11th day of drug treatment, tumour induction was carried out using DLA cells to Group I and II and EAC cells to Group III and IV. Ten days after, the measurement of tumour mass radii were taken, which was repeated on every 5th day for a period of 30 days. The volume of tumour mass was calculated from the formula given earlier.

Statistical analysis

Results are expressed as Mean ± SD and statistical analysis was carried out by using student's 't' test and analysis of variance (CRD) by one way classification.

RESULTS

Protein content

The protein content of crude extract, supernatant, flow through fraction and galactose eluted pooled sample were found to be1.9 g, 123 mg, 67 mg and 3.7 mg per ml respectively. The yield of the purified protein was 0.2% (Stock solution containing 3.7 mg abrin per ml).

HA and HI assays

Supernatant solution and purified abrin sample showed high HA titre values (1 : 512) and D-galactose (0.5 M) brought about inhibition of hemagglutination indicating the sugar specificity of the preparation.

Dose response of abrin

All the animals of Group VII and VIII died on the next day after the second dose abrin administration and only four animals survived after the final dose of abrin in Group VI. A dose of 1500 ng per animal was found to be the LD_{50} dose of abrin (75 µg/kg) after repeated experiments. Further experiments were done using 1/10th of the LD_{50} dose (7.5 µg/kg). Effect of abrin on mice receiving various concentrations of abrin on body weight and certain hematological parameters is given in Table I. On using abrin at the rate of 7.5 µg/kg per mouse on every alternate day for a total period of 10 days, it was found that the body weight which was reduced from 31.08 g to 29.93 g on 6th day and returned back to 31.58 g on 18th day, Hb content was reduced from 14.9 g% to 13.23 g% on 9th day and then returned back to 14.10 g% on 18th day, whereas RBC count was reduced from 8.83 millions/µl to 8.19 millions/µl on 9th day and returned back to 8.63 millions/µl on 18th day. There was significant changes in the body weight and hematological parameters of animals which received ½ LD_{50} dose (37.5 µg/kg) of abrin compared to other groups which received 1/10th and 1/20th of LD_{50} dose (Table I).

TABLE I : Dose response study in mice using abrin.

Days	*Body weight (g)*			*Hb concentration (g%)*			*RBC count (millions/µl)*		
	I	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
0	30.81±0.71[a]	31.08±0.44[a]	31.20±0.90[a]	14.60±0.43[a]	14.90±0.60[a]	14.63±0.65[a]	8.87±0.06[a]	8.83±0.28[a]	8.63±0.30[a]
3	30.41±0.44[b]	30.48±0.37[a]	31.04±0.40[a]	13.38±0.29[b]	14.15±0.21[a]	14.44±0.30[a]	8.36±0.39[a]	8.63±0.32[a]	8.39±0.33[a]
6	29.13±0.25[b]	29.93±0.49[a]	30.40±0.45[a]	12.61±0.69[b]	13.70±0.29[a]	13.98±0.29[a]	7.92±0.25[b]	8.29±0.33[a]	8.49±0.27[a]
9	28.51±0.67[b]	30.23±0.28[a]	31.01±0.58[a]	12.91±0.92[b]	13.23±0.37[a]	13.80±0.44[a]	7.48±0.32[c]	8.19±0.09[b]	8.50±0.27[a]
12	28.28±0.36[b]	30.43±0.33[a]	30.95±0.21[a]	12.31±0.67[b]	13.91±0.12[a]	14.13±0.32[a]	7.29±0.29[b]	8.28±0.27[a]	8.50±0.22[a]
15	29.08±0.50[b]	30.80±0.27[a]	30.96±0.48[a]	12.20±0.25[c]	14.14±0.16[b]	14.45±0.30[a]	7.72±0.14[b]	8.22±0.13[a]	8.52±0.23[a]
18	30.59±0.38[b]	31.58±0.32[a]	31.58±0.31[a]	13.41±0.35[c]	14.10±0.40[b]	14.50±0.15[a]	8.10±0.08[b]	8.63±0.24[a]	8.71±0.26[a]

Means bearing different superscripts differ significantly ($P<0.05$);
I-abrin administered @ 37.5 µg/kg on every alternate day for 10 days (5 doses);
II-abrin administered @ 7.5 µg/kg on every alternate day for 10 days (5 doses);
III-abrin administered @ 3.75 µg/kg on every alternate day for 10 days (5 doses).

Effect of abrin on the lifespan of ascites tumour bearing mice:

The average life span for untreated control animals (Group I) bearing DLA tumour cells was 50.2 ± 0.88 days and animals (Group III) bearing EAC tumour cells was 50.1 ± 1.1 days. The average life span of treated animals in group II bearing DLA tumour cells was 72.5 ± 1.6 days and that of group IV bearing EAC tumour cells was 69.8 ± 2.0 days. The life span of mice treated with abrin was found to increase by 44% for Group II animals and by 39% for Group IV animals.

EFFECT OF ABRIN ON SOLID TUMOURS

Simultaneous administration of abrin

Both intralesional and ip administration of 5 doses of abrin (75 µg/kg) was found to reduce the solid tumour development (Table II and III) significantly (P<0.01) when given simultaneously along with cancer cells. Intralesional administration of abrin (Group V) showed mean Ehrlich's tumour volume of 0.37 ml ± 0.03 on 30th day and the corresponding value of untreated controls (Group IV) was 3.00 ml ± 0.13. Similarly none of the animals in group II showed a measurable tumour mass on the site of treatment, which received abrin and DLA cells, when compared to their untreated controls (Group I) which showed a tumour volume of 2.89 ml ± 0.13. A tumour volume of 1.65 ml ± 0.07 induced by EAC cells and 1.82 ml ± 0.08 induced by DLA cells were recorded on 30th day for animals of Group III and VI, receiving abrin by ip route, which were found to be significantly lower (P<0.01) than the untreated control animals.

TABLE II: Effect of abrin on DLA cells induced solid tumour.

Methods of administration of abrin	*30th day tumour mass volume in ml (Mean±SD)*		
	Control	*Treated ip*	*Treated intralesionally*
Simultaneously with tumour cells	2.89±0.13[a]	1.65±0.07[b]	0.00±0.00[c]
After tumour development	2.89±0.13[a]	2.00±0.07[b]	1.09±0.01[b]

Means having different superscripts differ significantly (P<0.01).

TABLE III: Effect of abrin on EAC cells induced solid tumour.

Methods of administration of abrin	*30th day tumour mass volume in ml (Mean±SD)*		
	Control	*Treated ip*	*Treated intralesionally*
Simultaneously with tumour cells	3.00±0.13[a]	1.82±0.08[b]	0.37±0.03[c]
After tumour development	3.00±0.13[a]	2.74±0.13[b]	2.42±0.10[b]

Means having different superscripts differ significantly (P<0.01).

Effect of abrin on developed tumours:

Both intralesional and ip administration of 5 doses of abrin on the developed tumour mass was found to be effective in reducing tumour development (Tables II and III). The untreated control mice in Group I and IV showed a tumour volume of 3.00 ml ± 0.13 (induced by DLA cells) and 2.89 ml ± 0.13 (induced by EAC cells) respectively. Those animals of Group II and V which received abrin intralesionally showed a tumour volume of 1.09 ml ± 0.1 (DLA cells) and 2.42 ml ± 0.10 (EAC cells) as recorded on 30th day. Those animals which received abrin by ip route (Group III and VI) showed a tumour volume of 2.00 ml ± 0.07 (DLA cells) and 2.74 ml ± 0.13 (EAC cells) on 30th day. The developed tumour volume in mice of group II and V were significantly lower ($P<0.01$) when compared to untreated control animals.

Effect of administration of abrin prophylactically

Intraperitoneal administration of 5 doses of abrin prior to tumour cell inoculation was found to be less effective in controlling the tumour mass development (Table IV). The untreated control mice in Group I and III showed a tumour volume of 2.39 ml ± 0.14 (induced by DLA cells) and 2.56 ml ± 0.12 (induced by EAC cells) respectively. A tumour volume of 1.96 ml ± 0.09 and 2.2 ml ± 0.09 was recorded on 30th day for animals of group II and IV respectively, which received abrin by ip route. Tumour volume in mice of both these groups were lesser as compared to controls, though statistically it was not significant.

TABLE IV: Prophylactic effect of abrin on DLA and EAC cells induced solid tumours.

	30th day tumour mass volume in ml (Mean±SD)	
	Control	*Treated ip*
DLA cells induced tumour mass	2.39±0.14[a]	1.96±0.09[b]
EAC cells induced tumour mass	2.56±0.12[a]	2.20±0.09[b]

Means having different superscripts differ significantly ($P<0.01$).

DISCUSSION

Dose response studies showed that a dose of abrin at the rate of 75 µg/kg given on every alternate day fo 10 days (5 doses) was found to be a safe dose in mice. Abrin when used intralesionally was very effective, especially during simultaneous administration. Results revealed that on the developed tumour mass abrin brought about a marked reduction in tumour volume, while prophylactic use of abrin was unsatisfactory. Results also showed that when compared to EAC cells, the DLA cell line was more sensitive to abrin. The subunit A of abrin has got N-glycosidase and depurinating action of 28-S RNA of ribosome, thereby arresting protein synthesis (15). Inhibition of protein synthesis induces programmed cell death or apoptosis regardless of the mechanism of action of the inhibitor (16) could be the reason for the tumouricidal action of abrin. An overall increase in life span of ascites tumour bearing mice treated with abrin was quite significant and this finding is in agreement with an earlier report (17).

Lectins are highly sugar specific and some of them are used in differentiatial staining of tumour cells from the normal. Tumour cells exhibit more glycosylated binding sites over their surface than normal cells (18, 19). Epstein Barr Virus transformed lymphocytes showed 57000 to 360000 abrin receptors per cell (20). The B subunit of abrin binds to cell surface receptors containing terminal galactose in glycolipids as well as glycoproteins and thereby facilitates endocytosis and subsequent translocation of subunit A into cytosol. Binding of atleast 3000–10000 abrin molecules per Chinese Hampster ovary cell was required to elicit 50% loss of cell viability (21). Presence of abundantly available glycosylated binding sites and requirement of comparatively lesser number of abrin molecules for initiating cell death could be one of the reasons why abrin in nanogram concentrations bring about tumouricidal effect especially when it was delivered directly into the tumour mass which facilitated direct binding of abrin with tumour cells before getting diluted by other fluids of body system.

Like abrin, ML-I and ML-II lectins from *Viscum album*, are also β-galactose specific lectins, extracts from *Viscum album* exhibited effective antitumour activity, antimetastatic and anticarcinogenic properties (22, 23). It has been reported earlier that β-galactoside specific lectin mediated immunomodulation was found to be antitumoral and antimetastatic activity in experimental animals (24). Whether there is a possible role of immunomodulatory effect of abrin in host's immune system is yet to be ascertained for the antitumour activity of abrin.

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