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Tumour reducing activity of an isolated active ingredient from mistletoe extract and its possibile mechanism of action

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A peptide of approximate molecular weight 5000 was isolated in our laboratory from Mistletoe extract (Iscador) using chromatographic method. This isolated material at a concentration of 10^{-7} M was found to produce 100% cytotoxicity to KB cells, Chinese Hamster ovary cells, LB cells, Dalton's lymphoma ascites tumour cells and Ehrlich ascites tumour cells. The isolated material at concentrations of 2.5 mg/kg body weight reduced the growth of solid tumours induced in mice by Dalton's lymphoma ascites cells (DLA-cells). The tumour reducing action of the isolated material could be seen when the drug was given either along with the cells, after tumour development or when given prophylactically. A «receptor» for this peptide was found in the cell sonicates of Dalton's lymphoma cells as well as in the ascites fluid which inhibited the cytotoxicity of the isolated component. However the receptor was not seen in the cell sonicates of lymphocytes which are not susceptible to the cytotoxic action of the isolated component indicating that the binding of the active ingredient in the Iscador to the cell surface receptor is needed for its action. Initial studies indicated that the isolated peptide is a complex molecule and separation of the individual component produced a loss of activity.

Key Words: Tumour activity, Mistletoe extract.

A preparation from Mistletoe (Viscum album) known by the proprietory name «Iscador» had been introduced as an immune stimulating drug against cancer. Use of this extract in increasing the survival rate in many types of cancer patients has been documented (2, 10). Moreover administration of this extract has been reported to reduce post-operative recurrence of certain tumours (11, 12).

Recently we have isolated a cytotoxic and tumour reducing component from Iscador which is a peptide of 5000 molecular weight (9). In the present manuscript we have determined the activity of the isolated component from Iscador on solid tumours produced by Dalton's lymphoma ascites tumour cells under various modalities of drug treatment. Moreover we have partially characterized the peptide and have given evidence to suggest that the binding of the active ingredient from Iscador to a cell surface receptor is needed for producing its action.

Materials and Methods

Iscador used in this study was a proprietory injectable preparation from Mistletoe (Viscum album) made by Varein Fuer Krebsforschung, Switzerland and the authors are grateful to Dr. M. Werner for supplying these samples. Iscador M (5%) used in this study is a soluble, sterile, aqueous extract from Viscum album and the concentration of crude fresh plant is 5 gms/100 ml.

In vitro cytotoxic studies using Dalton's lymphoma ascites tumour cells (DLA-cells) and human lymphocytes (prepared by Ficoll-Hypaque method) were done using trypan blue exclusion method (8).

Isolation of the tumour reducing component from Iscador

Isolation of the tumour reducing peptide from Iscador was done by the method already described by Kuttan et al (9). Fast protein liquid chromatographic analysis of the isolated sample was done using a Pharmacia FPLC instrument using Mono-Q, Mono-S and Sepharose-12 column. The separation of the isolated material was done by a sodium chloride gradient (0.1 M) in Tris-HCl (pH 7.4).

Animal experiments

Inbred strains of Swiss albino mice (16-20 gms) (six in each group) where used for each set of animal experiments. Dalton's lymphoma ascites tumour cells (1 million in 0.1 ml) were injected subcutaneously on the hind limb of the animals. Isolated peptide (10 μg in 0.1 ml saline) was injected to each animal subcutaneously using a 26 gauge needle at the same limb where the tumour had been transplanted to determine its effect on solid tumour development. The controls received 0.1 ml saline solution. Injections were administered for (A) five alternate days after 24 hrs of administration of cells according to the standard protocol; (B) five alternate days before the administration of cells, to determine the prophylactic action of the isolated component against tumour development; (C) ten alternate days, six days after the administration of cells to find out the action of the isolated component on developed tumours. Solid tumours were measured 7 days after the injection of cells and measured every three days thereafter. Tumour volume was determined from the diameter as described earlier (8).

Table I - Cytotoxicity of isolated material to various cell lines

Cell line	Concentration needed for 100% cell death (μg/ml)
Dalton's lymphoma cells	2.2
Ehrlich ascites cells	1.1
Sarcoma-180 cells	0.44
Chinese hamster ovary cells	2.2
Lymphocytes (human)	> 44.4
Lymphocytes (leukemia patients)	> 44.4

Assay of receptor for the isolated component in the cell sonicate

Assay is based on the observation that when the isolated component is mixed with a sonicate of receptor positive cell it loses its cytotoxic activity. A cell sonicate of 10^7 cells/ml of Dalton's lymphoma cells and lymphocytes were prepared in saline. The isolated component (10 μg) was mixed with a serially diluted solution of the sonicate (0.1 ml) and volume was made up to 0.9 ml. Dalton's lymphoma cells (10^6 in 0.1 ml) were added and incubated for 3 hrs at 37°C. After the incubation the percentage of dead cells was determined by trypan blue exclusion. The receptor activity in the cell sonicate was expressed as the highest dilution in which the inhibition of cytotoxic activity could be observed under the conditions studied.

Results

Cytotoxicity of the isolated peptide

Various cell lines were found to be susceptible to the cytotoxic action of the isolated peptide from Iscador (Table I). This included Dalton's lymphoma ascites tumour cells, Ehrlich ascites tumour cells, Sarcoma-180 tumour cells and Chinese hamster ovary cells. The concentration needed for 100% cytotoxicity was found to be 0.4-2 μg/ml during the short term incubation. The cyto-

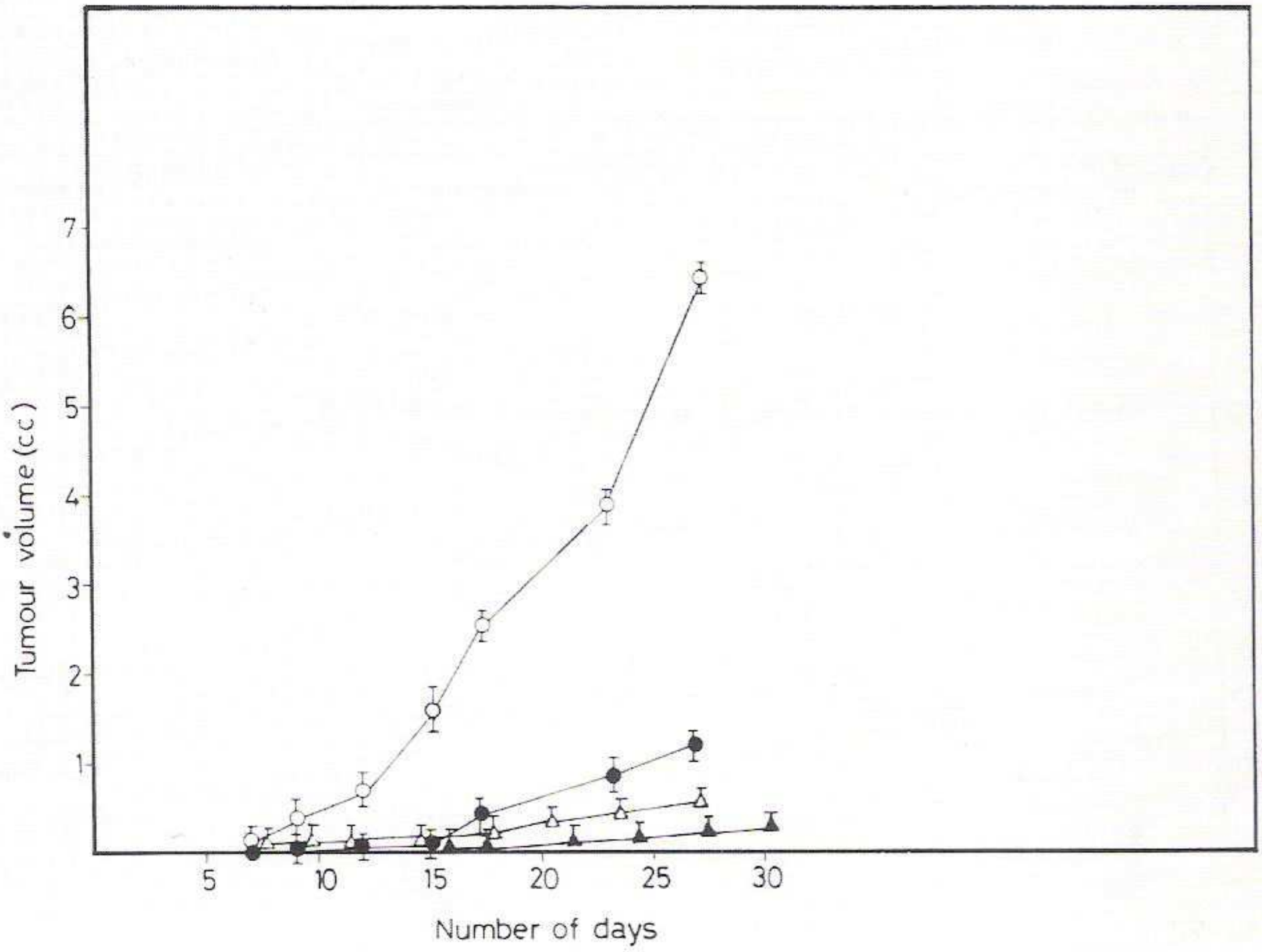

Fig. 1 - Effect of the isolated material given under various modalities of treatment on solid tumour development.
○—○ Untreated animals. ▲ — ▲ Treated with the drug prophylactically.
△ — △ Treated with the drug 24 hrs after the injection of cells.
▲ — ▲ Treated with the drug 6 days after injection of tumour cells.

toxic activity was found to be increased when the cells were in contact with the isolated component for prolonged periods of time in the tissue culture.

Tumour reducing activity of the isolated peptide

The isolated peptide was found to significantly ($P < 00.1$) reduce the solid tumours induced by Dalton's lymphoma ascites tumour cells in mice. The tumour reducing activity was seen when the drug was given during the tumour transplantation, prophylactically or after the development of tumour (Fig. 1).

Characterization of the isolated peptide

FPLC analysis of the isolated peptide using a molecular sieve column produced a wide peak with a molecular weight of approximately 5.000. The peak was not homogenous indicating that the material may be a mixture of more than one component.

Analysis of Mono-S column (cation exchanger) indicated that the material was not found attached to the column and was eluted in the break through fraction.

However, analysis using Mono-Q column (anion exchanger) produced two peaks which appeared in early gradient and two peaks in

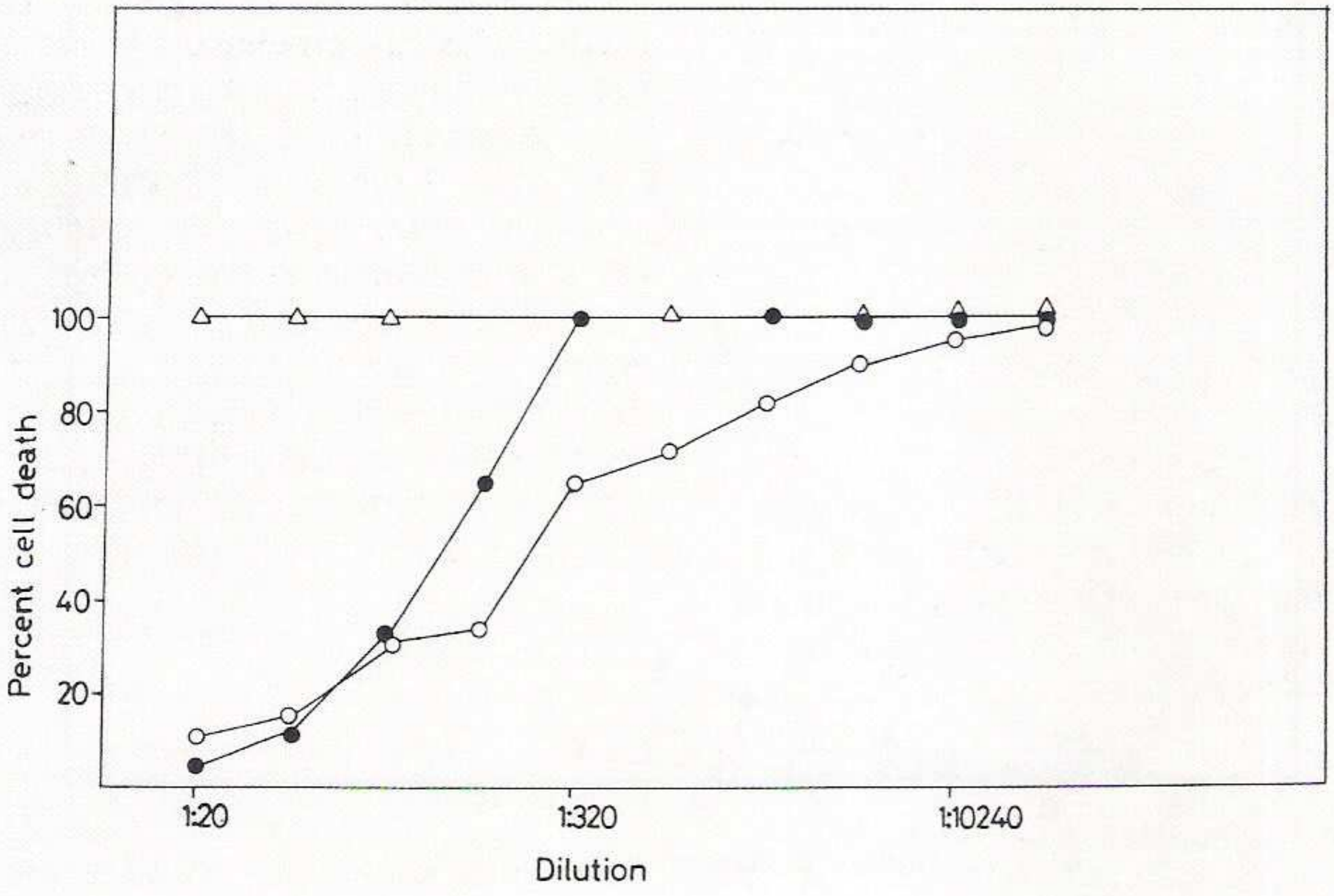

Fig. 2 - Inhibition of cytotoxicity of the isolated component by cell sonicates.
●—● Dalton's lymphoma cell sonicate. ○—○ Ehrlich ascites cell sonicate.
△ — △ Sonicate of normal human lymphocytes.

the higher gradient fractions. This experiment indicated that the peptide may be either neutral or acid but not basic.

The activity of the fractions were checked (Table II). It was observed that the activity of the isolated peptide was considerably lost upon separation by FPLC as none of the fractions, excepting fraction I, showed any considerable activity both *in vitro* as well as in culture. Similarly their tumour reducing activity was also considerably low (data not shown).

Role of a receptor for the activity of the isolated peptide on cells

When the isolated component was pretreated with a sonicate of Dalton's lymphoma cells, its cytotoxic activity was considerably reduced both in the short term experiment as well as in tissue culture studies (Fig. 2). The amount of the sonicate needed to inhibit the activity of the peptide was very small, indicating a receptor type of mechanism.

Discussion

The data presented here indicates the presence of peptide of approximate 5000 molecular weight in Iscador which inhibited the experimental tumours in animals. This peptide was also found to be cytotoxic to several cells in culture. However cells which are lymphocytic in origin are not susceptible to the action of the peptide. Moreover these cells are found to have proliferated in

Table II - Growth inhibition of KB-cells by isolated fractions from Mono-Q column

Fraction number	Cell growth (%) of control
I	3.5%
II	37 %
III	100 %
IV	71.4%

the presence of the peptide (G. Kuttan and R. Kuttan, in press). The isolated peptide was also found to inhibit the tumour growth when given prophylactically, indicating that this peptide component is responsible for the tumour reducing and immunological activity of the Iscador which is represented here as well as in Broksma et al. and in Hajto et al. (1, 4).

Several active components with tumour reducing activity have been isolated from Viscum album (3, 7, 13). The reported molecular weight of some of these components were high (5, 15). Moreover, it has been noted that the tumour reducing component is a complex protein molecule and extensive purification produced a loss of activity (14). Our results indicated that the active component is a complex molecule of approximately 6000 molecular weight, the separation of which leads to the inactivation. Moreover FPLC data does not indicate the presence of Viscotoxin in this samples as reported by Jung et al. (6) as Viscotoxin could be expected to attach to the Mono column.

The data reported here also indicates the presence of a receptor molecule on the cells which are sensitive to the isolated peptide. The binding with the receptor reduces the cytotoxic activity of the isolated peptide both in the short time incubation as well as in tissue culture using KB cells. Receptor molecule was heat inactivable and could be separated on Sephadex column. As many proteolytic enzymes had only limited activity, the loss of activity of the isolated peptide is not due to a proteolysis, however, non-specific binding could not be excluded.

From the complexity of the isolated peptide and its binding characteristics it is logical to assume that one portion (lectin?) produced specific binding on the cell and the other portion produced cytotoxicity. The peptide was also found to produce several of the immunomodulating activities, inducing monocyte maturation, proliferation, macrophage activation and macrophage induced cytotoxicity (G. Kuttan & R. Kuttan-unpublised). These activities could be mediated by the lectin molecule. Moreover it is quite logical to assume that the activity is mediated through interferon or other immunomodulatory materials present in the cells.

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