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EFFECT OF A PREPARATION FROM *VISCUM ALBUM* ON TUMOR DEVELOPMENT IN VITRO AND IN MICE

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Summary

The effect of Iscador, a commercial preparation made from *Viscum album* was studied on several cell lines using in vitro tissue culture as well as tumor-bearing animals. Iscador was found to be cytotoxic to animal tumor cells such as Dalton's lymphoma ascites cells (DLA cells) and Ehrlich ascites cells in vitro and inhibited the growth of lung fibroblasts (LB cells), Chinese hamster ovary cells (CHO cells) and human nasopharyngeal carcinoma cells (KB cells) at very low concentrations. Moreover, administration of Iscador was found to reduce ascites tumours and solid tumours produced by DLA cells and Ehrlich ascites cells. The effect of the drug could be seen when the drug was given either simultaneously, after tumour development or when given prophylactically, indicating a mechanism of action very different from other chemotherapeutic drugs. Iscador was not found to be cytotoxic to lymphocytes.

Introduction

Mistletoe (*Viscum album* L., family: Loranthacea) is a plant considered to have several medicinal properties in the folklore of Europe (Steiner, 1920). A preparation from *Viscum album* known by the name Iscador is reported to be immunostimulating and is being used in Europe for cancer therapy (Vestar et al., 1968; Evans and Preece, 1973; Leroi, 1975). Neoplasms reportedly responding to Iscador therapy include breast cancer, bladder cancer, melanoma, ovarian cancer and reticulosarcoma (Leroi, 1975). Interestingly, Iscador was found to be effective when given prophylactically, hence its use in reducing recurrence of tumours after surgery when radiation treatment has been suggested (Salzer and Harlec, 1978). However, certain tumours and cell lines were not found to be sensitive to Iscador therapy (Berger and

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Schamhl, 1983). Leukemias generally do not respond to Iscador therapy; the reason for the resistance is not known at present.

In the present manuscript, the effects of Iscador on various cells both in vitro and in tissue culture have been studied and an attempt has been made to correlate the cytotoxicity with the tumour reduction produced in animals. Moreover, Iscador was administered under various modalities and the comparative effects assessed.

Materials and methods

Plant material

Iscador is a sterile injectable preparation from *Viscum album* manufactured by Varein für Krebsforschung (Arlesheim, Switzerland). Iscador M (5%) prepared from *Viscum album* grown on apple trees was used in this study. It is a soluble aqueous extract from the whole plant and equivalent to 5 g of fresh plant material per 100 ml.

In vitro studies

Cytotoxic studies using Dalton's lymphoma cells, Ehrlich ascites cells and human lymphocytes (prepared by the Ficol; Hypaque method) were carried out by incubating the cells with various concentrations of the drug at 37 °C for three hours and determining cytotoxicity by the trypan blue exclusion method as described elsewhere (Kuttan et al., 1985). The tissue culture experiments were done using lung fibroblasts (LB cells), human nasopharyngeal carcinoma cells (KB cells) and Chinese hamster ovary cells (all obtained from the National Institute of Virology, Poona) grown in Minimum Essential Medium (Eagle) and 10% calf serum. These cells (2×10^4) were grown in the presence of various concentrations of Iscador in triplicates for 5 days and inhibition of cell growth was determined by counting the viable cells using a haemocytometer.

In vivo studies

Groups of six Swiss albino mice (16—20 g) were used for animal experimentation. They were housed in ventilated cages and fed with mouse chow (Lipton, India) and water ad libitum. Tumour cells (1×10^6) were transplanted into mice intraperitoneally to develop as ascites tumours and injected subcutaneously in a hind limb to form solid tumours. Iscador-M (5%) was diluted 1 to 3 in saline and 0.1 ml injected to each animal either i.p. (in ascites tumour) or i.m. (in the same limb as the solid tumour). This amount is equivalent to 83 mg of mistletoe/kg body weight. Injections were done: (*i*) on five alternate days, 24 h after administration of cells according to the standard protocol; (ii) on five alternate days before the administration of cells to determine the prophylactic potential of Iscador and (*iii*) on 10 alternate days 6 days after the administration of cells to establish the action of Iscador on developed tumours. Solid tumours were measured 7 days after the injection

of cells and measured every three days thereafter. The tumour volume was calculated from the formula $V = 4/3 \cdot \pi \cdot r_1^2 \cdot r_2^2$ were r_1 and r_2 are two independent measurements of the tumour radius. In the case of ascites tumours, the survival of animals was determined 30 days, 45 days and 60 days after the injection of cells and the increase in life span was calculated relative to untreated animals.

Results

Cytotoxicity of Iscador

Iscador was found to be cytotoxic to various cells both in vitro where the incubation time was only 3 h as well as in tissue culture (5 days) (Table 1). The concentration of Iscador needed for 50% cell death in DLA cells and Ehrlich ascites cells was found to be 10—20 μl/ml, which was approximately equal to 0.5—1.0 mg of fresh plant material. Iscador was needed in much lower concentrations (1—9 μl/ml) to inhibit the growth of cells in tissue culture. Iscador was not found to be cytotoxic to normal lymphocytes, lymphocytes from lymphoblastic leukemia patients and lymphocytes isolated from mouse spleen cells. Normal lymphocytes after activation with phytohaemagglutinin (PHA) did not respond in a cytotoxic manner to Iscador treatment indicating a specificity of the action of Iscador to certain cells.

Inhibition of ascites tumour development in mice

The effect of Iscador on ascites tumour development was studied using

TABLE 1

CYTOTOXICITY OF ISCADOR TO VARIOUS CELL LINES

Cell line	Minimum concentration needed for 50% cytotoxicity or 50% inhibition of cell growth (μl/ml)
Chinese hamster ovary cells[a]	7
KB cells[a]	1
LB cells[a]	1
Dalton's lymphoma cells	20
Ehrlich ascites cells	10
Normal lymphocytes (human)	>200
Normal spleen cells (mouse)	>200
Lymphocytes (leukemia patients)	>200
Lymphocytes after activation with PHA	>200

[a]Activity was determined in tissue culture.

TABLE 2

EFFECT OF ISCADOR ON ASCITES TUMOURS IN MICE

Tabular values are the mean ± S.E.M. of six animals in each group. Iscador (33 μl) was given from day 1 to day 10 on alternate days.

Type of tumour	Days animals survived (*N*)	Increase in life span (%)
Dalton's lymphoma		
Non-treated	20.0 ± 7.8	
Treated	31.0 ± 1.0	55.0*
Ehrlich		
Non-treated	11.5 ± 1.5	
Treated	20.2 ± 7.1	75.6*

Significance from non-treated: *$P < 0.01$.

Dalton's lymphoma ascites tumour and Ehrlich ascites cells. The results are shown in Table 2. Administration of Iscador along with tumour cells significantly increased the survival of tumour bearing mice by more than 50%.

Effect of Iscador on solid tumour development

Injection of Iscador had a remarkable effect in reducing the development of solid tumour in mice induced by Dalton's lymphoma ascites cells and Ehrlich ascites cells. This effect could be demonstrated when the drug was given simultaneously with tumour cells, after tumour development and prophylactically (Table 3).

Simultaneous injection of Iscador along with tumour cells considerably reduced the tumour volumes produced by Dalton's lymphoma ascites tumour and Ehrlich ascites tumour. The tumour volume on day 30 was 12% that of control in the case of Dalton's lymphoma tumour and only 3.6% that of controls in the case of Ehrlich ascites tumour. These differences are very highly significant.

Iscador was found to have significant effects when given after tumour development. Tumour volume on day 30, was 21% of controls in the case of Dalton's lymphoma ascites tumour and 10% in the case of Ehrlich ascites tumour.

Similarly, prophylactic administration of Iscador was found to reduce tumour development. Animals treated prophylactically did not develop tumours as in the controls. Tumour volume on the 30th day was 24% of control in the case of Dalton's lymphoma tumour and 2.7% in the case of Ehrlich ascites tumour. These differences were very highly significant.

Injection of Iscador did not significantly reduce body weight in these

TABLE 3

EFFECT OF ISCADOR ON SOLID TUMOUR DEVELOPMENT IN MICE

Results represent the mean ± S.E.M. of 6 animals/group. Iscador given was 33 μl/animal. For the individual treatment schedule see Materials and methods section of the text.

Type of tumour	Treatment schedule	Tumour volume (ml)		
		+ 20 days	+ 30 days	+ 40 days
Dalton's lymphoma	Non-treated	0.965 ± 0.248	2.480 ± 0.534	5.230 ± 1.223
	Treated simultaneously	0.200 ± 0.121***	0.300 ± 0.232***	0.600 ± 0.432***
	Treated after 7 days of tumour development	0.300 ± 0.240*	0.530 ± 0.532*	0.700 ± 1.050*
	Treated prophylactically	0.180 ± 0.082***	0.600 ± 0.054***	1.100 ± 1.000***
Ehrlich	Non-treated	0.700 ± 0.753	1.300 ± 1.116	5.000 ± 1.504
	Treated simultaneously	0.038 ± 0.048***	0.047 ± 0.065***	0.064 ± 0.124***
	Treated after 7 days of tumour development	0.063 ± 0.047***	0.133 ± 0.172***	0.195 ± 0.280***
	Treated prophylactically	0.015 ± 0.006***	0.036 ± 0.039***	0.038 ± 0.040***

Statistical significance: *$P < 0.05$, **$P < 0.001$.

animals. Moreover, Iscador did not produce bone marrow suppression since white blood cell counts were not reduced. In fact, Iscador has been shown to stimulate the WBC production in experimental animals (Rentea et al., 1981). Increasing Iscador concentration by 10 times did not produce any significant changes in body weight and did not show any additional advantage in suppressing tumour development.

Discussion

Iscador, a commercial preparation from European mistletoe, was found to be potentially active against tumour development using two tumour cell lines both as ascites and solid tumours. Iscador reduced the growth of already developed tumours in these animals and had prophylactic activity towards tumour development. The prophylactic activity of Iscador has been reported in patients undergoing Iscador therapy (Salzer and Harlec, 1978). Thymocyte stimulation has been reported to be one of the reasons for this effect.

The concentration of Iscador needed for activity was very low as compared to other chemotherapeutic drugs. Very recently, a cytotoxic peptide was found to be present in Iscador which was shown to reduce tumour growth in mice (Kuttan et al., 1988). The concentration of this component was estimated to be 3.7 mg/100 ml of Iscador.

It is noteworthy that Iscador did not have any cytotoxicity for human lymphocytes, either control or stimulated. Iscador has been shown to increase the stimulation mediated by phythohaemagglutinin and concanavalin-A (Bloksma et al., 1982). This suggests that Iscador therapy does not harm the haematopoietic system which is very common with other chemotherapeutic drugs. The non-effectiveness of Iscador to lymphoblastic leukemia cells (P-388) and lymphocytic leukemia (L-1210), therefore, may be explainable since these cells are of lymphocytic origin (Berger and Schambl, 1983). Iscador has been reported to be useful in treating lymphocytopenias associated with radiation therapy but not effective against leukemia (Dr. Leroi, personal communication).

Our preliminary data regarding the cytotoxic principle in Iscador indicate that its cytotoxicity is not destroyed by proteolysis and heat denaturation and its Sephadex filtration profiles are indicative of a 5000 moelcular weight component (Kuttan et al., 1988). Other investigations have suggested that the cytotoxic principle may be lectin (Franz, 1984) or an alkaloid (Khwaja et al., 1986). It seems that the active component present in Iscador may bind with a cell surface protein and produce its effect by inhibition of mitosis (Gayon et al., 1986). However, the exact mode of action of Iscador is not known at present.

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