Prevention of 20-methylcholanthrene-induced sarcoma by a mistletoe extract, Iscador

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Iscador, an extract from the semi-parasitic plant *Viscum album,* was found to inhibit 20-methylcholanthrene-induced carcinogenesis in mice. Intraperitoneal administration of Iscador (1 mg/dose) twice weekly for 15 weeks could completely inhibit 20-methylcholanthrene-induced sarcoma in mice and protect these animals from tumour-induced death. Iscador was found to be effective even at lowered doses. After administration of 0.166, 0.0166 and 0.00166 mg/dose 67, 50 and 17% of animals respectively did not develop sarcoma.

Introduction

Carcinogenesis involves multistage cellular processes (1,2). The first step is initiation, in which the carcinogen causes mutational changes in cellular chromosomes, followed by promotion, during which several oncogenic genes are transcribed with a marked increase in the protein products, such as protein kinases, growth factor receptors and growth factors (3). If these steps which lead to neoplastic growth could be inhibited formation of tumours could be prevented.

Iscador, prepared from the semi-parasitic plant *Viscum album,* has been found to be clinically useful in several neoplastic conditions (4,5). Iscador was reported to inhibit growth of transformed cell lines in culture (6) and to retard growth of animal ascites and solid tumours (7). The active material present in Iscador was reported to be a peptide of molecular weight 5000 (8). Cytotoxic lectins MLI and MLII also contribute to the biological activity of Iscador (9).

Iscador and its active ingredients have been reported to stimulate humoral and cell-mediated immune responses (10–12), considered to be one of the mechanisms of action of Iscador. In the present paper we show for the first time that Iscador can effectively inhibit methylcholanthrene-induced sarcoma at very low concentrations in mice, indicating that this extract may be highly relevant in chemoprevention.

Materials and methods

20-Methylcholanthrene was purchased from ICN Pharmaceuticals (New York, NY). Iscador (batch no. Ch-B-3121) was purchased from Weleda AG (Arlesheim, Switzerland). It was supplied as sterile injectable ampoules containing 5% aqueous extract of *Viscum album.* All other chemicals used were of analytical reagent grade. Swiss albino mice were purchased from the Animal Facility at the National Institute of Nutrition (Hyderabad, India). The animals were maintained in air-conditioned rooms and fed with normal mouse chow (Lipton, India) and water ad libitum.

Effect of *Viscum album* extract on development of 20-methylcholanthrene-induced sarcoma

Female Swiss albino mice (6–8 weeks old, 20–25 g) were randomly grouped as treated (n = 15) and non-treated (n = 15). Hair from the dorsal side of the animals was removed 24 h before the experiment. A single dose of 200 |g of 20-methylcholanthrene in 0.1 ml dimethyl sulphoxide was administered s.c. on the dorsal surface of each animal. *Viscum album* extract (Iscador) was administered i.p. twice a week (1 mg plant material/dose) for 15 weeks on Mondays and Thursdays in the morning. Control animals were treated with the same volume (0.1 ml) of isotonic saline. The animals were observed for 6 months for development of sarcoma and survival.

**Effect of different dilutions of Iscador on 20-methylcholanthrene-induced sarcoma development and survival**

Female Swiss albino mice (6–8 weeks old, 20–25 g) were randomly grouped as treated (n = 10/group, five groups) and non-treated (n = 10). All animals were treated with a single administration of 20-methylcholanthrene (200 µg/ mouse) on the dorsal side. Iscador was diluted with isotonic saline and each group of animals was treated with different dilutions of Iscador containing various concentrations of the plant material ranging from 1.66 to 0.00166 mg/dose/animal for 15 weeks. Control animals were treated with the same volume (0.1 ml) of isotonic saline. The animals were observed for the onset of sarcoma and survival for 6 months.

Results

**Effect of Iscador on development of chemically induced sarcoma**

The effect of *Viscum album* extract (Iscador) on development of sarcoma induced with methylcholanthrene is shown in Table I. The animals in the control group started developing sarcomas 40 days after carcinogen administration, whereas in the Iscador-treated group none of the animals developed sarcomas up to 120 days. Only one animal developed sarcoma 140 days after carcinogen administration. The size of the tumour in this animal was found to be very small compared with that of the control animals. There was no difference in food consumption and body weight of the different groups of animals when compared with that of the tumour-bearing mice, indicating that caloric intake did not influence the difference in tumour burden.

Histopathological analysis of the tumour indicated it to be a fibrosarcoma, which has also been reported by other investigators (13). Since all untreated animals died, the cause of death could be considered as due to tumour burden. Metastasis of the tumour to other sites was not considered in this experiment.

**Effect of Iscador administration on the survival of animals treated with methylcholanthrene**

The effect of *Viscum album* extract (Iscador) on survival of animals injected with 20-methylcholanthrene is given in Table II. The animals in the control group started dying due to tumour burden after 80 days of methylcholanthrene treatment. All the animals in the Iscador-treated group were alive even after 180 days of methylcholanthrene treatment. Except for one animal which developed sarcoma, all other animals were found to be healthy when the experiment was completed.

**Effect of different dilutions of Iscador on survival and sarcoma development**

The effect of administration of different dilutions of Iscador containing various amounts of *Viscum album* extract on sar-
Moreover, its usefulness in inhibiting the recurrence of tumours in experimental animals. The potent anticancer activity of *Viscum album* preparation (Iscador) was previously known (6,7).

The results presented in this manuscript indicate the usefulness of Iscador in inhibiting chemically induced carcinogenesis in mice treated with methylcholanthrene. A single dose of 20-methylcholanthrene (200 µg/0.1 ml/mouse) was injected s.c. on the dorsal side. Iscador was given i.p. (1 mg/0.1 ml/mouse) twice weekly for 15 weeks. The mice were observed for 6 months.

### Table I. Effect of *Viscum album* extract on sarcoma development

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals developing sarcoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8/15 8/15 15/15 0/15 0/15 0/15 0/15 1/15 1/15 0/15</td>
<td>15/15</td>
</tr>
<tr>
<td>Iscador</td>
<td>0/15 0/15 0/15 0/15 1/15 1/15 1/15 1/15 1/15 0/15</td>
<td>1/15</td>
</tr>
</tbody>
</table>

A single dose of 20-methylcholanthrene (200 µg/0.1 ml/mouse) was injected s.c. on the dorsal side. Iscador was given i.p. (1 mg/0.1 ml/mouse) twice weekly for 15 weeks. The mice were observed for 6 months.

### Table II. Effect of *Viscum album* extract on survival of mice treated with methylcholanthrene

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals surviving</th>
<th>No. dying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15/15 15/15 15/15 2/15 0/15 15/15 15/15 15/15 15/15 0/15</td>
<td>15/15</td>
</tr>
<tr>
<td>Iscador</td>
<td>15/15 8/15 5/15 2/15 0/15 15/15 15/15 15/15 15/15 0/15</td>
<td>1/15</td>
</tr>
</tbody>
</table>

A single dose of 20-methylcholanthrene (200 µg/0.1 ml/mouse) was injected s.c. on the dorsal side. Iscador was given i.p. (1 mg/0.1 ml/mouse) twice weekly for 15 weeks. The mice were observed for 6 months.

### Table III. Effect of various dilutions of *Viscum album* extract on sarcoma development

<table>
<thead>
<tr>
<th>Dilution</th>
<th><em>Viscum album</em> injected/dose</th>
<th>Animals developing sarcoma (%)</th>
<th>Animals surviving to 20 weeks (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:3</td>
<td>0.00166 mg</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1:5</td>
<td>0.166 mg</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>1:30</td>
<td>166 µg</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>1:300</td>
<td>16.6 µg</td>
<td>50</td>
<td>17</td>
</tr>
<tr>
<td>1:3000</td>
<td>1.66 µg</td>
<td>83</td>
<td>17</td>
</tr>
</tbody>
</table>

A single dose of 20-methylcholanthrene (200 µg/0.1 ml/mouse) was injected s.c. on the dorsal side. *Viscum album* extracts were administered i.p. twice weekly for 15 weeks.

The use of Iscador to inhibit chemically induced carcinogenesis has been reported.

The mechanism of action of Iscador has mainly been attributed to stimulation of both cell-mediated and humoral immunity (12). Iscador has been shown to stimulate T cells (15) and to increase the production of cytokines such as IFN (16), IL-1 (9) and TNF (17,18). The present results showing that Iscador can inhibit chemical carcinogenesis at very low concentrations indicate that: (i) the increased immune response produced by administration of Iscador may be the reason for inhibition of growth of neoplastic cells; (ii) Iscador may be preventing transformation of neoplastic cells by inhibiting activation of oncoproteins and further signal transduction pathways; (c) Iscador may be able to reverse transformation or specifically inhibit growth of transformed cells.

It is known that Iscador is more cytotoxic to transformed cells than to non-transformed cells (7). This is also consistent with the observation that a cellular component (a receptor) present in transformed cells could bind the active peptide present in Iscador (19). It could be postulated from these observations that the active component in Iscador may retard the growth of transformed cells by inhibiting some of the cellular events that lead to cell proliferation. The mechanism of action of the extract may be related to its specific reversal/inhibition of the oncogene activation cascade.

### References

Anticarcinogenic action of mistletoe extract


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