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Presence of a receptor for the active component of Iscador in ascites fluid of tumour bearing mice

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Summary

Tumour bearing mice exhibit a specific "receptor" in the ascites fluid which binds with the active component isolated from Iscador. This "receptor" was found to be a protein which inhibited the cytotoxicity of Iscador and its isolated active component at low concentration. The receptor protein was also found in the sonicates of tumour cells which are susceptible to the action of Iscador but not in lymphocytes which were not susceptible to Iscador or its isolated active component. The receptor was separated on a Sephadex G-50 column. Activity was lost upon heat denaturation and dialysis.

Keywords: iscador; mistletoe; receptor; anti-tumour drugs.

Introduction

A preparation from *Viscum album* known as Iscador has been shown to produce considerable activity against several neoplasms in both humans and animals [2,4,7,10]. While several tumours were found to be susceptible to the action of Iscador, some tumours both humans and animals do not respond to the Iscador therapy [1,4,9]. For example, it has been observed that Iscador could not be used for the treatment of leukemias (Leroi, pers. commun.) and that the P388 and L1210 leukemia models in animals do not respond to Iscador administration [1,4].

Recently we identified a tumour-reducing component present in Iscador [5,6]. This component was found to be a cytotoxic peptide of molecular weight of approximately 5000. This component was found to be cytotoxic to several cells in vitro and in tissue culture at approximate concentrations of 10^{-7} M, but was not cytotoxic to lymphocytes form normal persons and from patient with leukemia indicating a cell specificity. In the present work we show the presence of a "receptor" in the susceptibile tumour cells sonicates as well as in the ascites fluid of tumour bearing mice but not in lymphocytes.

Materials and methods

Iscador M (5%) used in this study was a gift from late Dr. Rita Leroi, Varein Fuer Krebsforschung, Switzerland, which was supplied as sterile injectible ampules. The active component of Iscador used in this study was parpared by the procedures which has been already described elsewhere [6]. All other chemicals

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used in this study were of analytical reagent grade.

Animals

Female Swiss albino mice (20—25 g) were used for all the experiments. They are housed in ventilated cages and fed with normal mouse chow and water ad libitum. Dalton's lymphoma asictes tumour and Ehrlich ascites tumour were grown in the peritoneal cavity of these mice by transplanting 1×10^6 cells. Ascites fluid were collected during the later stages of tumour development by aspiration.

Assay of receptor activity

The receptor activity was measured by the inhibition of the cytotoxic activity of Iscador to tumour cells. Ascites fluid of tumour bearing mice was collected by aspiration. It was immediately centrifuged at 1000 rev./min for 10 min, and the supernatant was used for the assay. In a typical assay a serially diluted ascites fluid (100 µl) was mixed with respective tumour cells (1×10^6) and diluted to 0.8 ml with phosphate buffered saline and mixed with Iscador M (5%) (100 µl) or isolated component (25 µl equivalent to approx. 10 µg) to a final volume of 1 ml and incubated for 3 h at 37 °C. After incubation the viability of cells was determined by the Trypan Blue exclusion method. Receptor activity was expressed as the dilution at which inhibitory activity was maximally reversed.

Sonicates of tumour cells (10^6 cells/ml) were prepared using a Labline sonicator. Lymphocytes from normal persons were separated by the Ficoll-Hypaque method and these cells (after washing) were sonicated as described above. Sonciates (0.1 ml) were used in place of the ascites fluid in the experiments to determine receptor activity as described above.

Sephadex G-50 column (40 cm × 2 cm) chromatography of the ascites fluid from Ehrlich ascites tumour was performed using 0.3 ml ascites fluid mixed with 0.7 ml phosphate buffered saline and with phosphate buffered saline as the elutent. Two-millilitre fractions were collected. Fractions (0.5 ml) were assayed for the inhibition of cytotoxic activity produced by Iscador to Ehrlich ascites cells as given above.

Results

The Dalton's lymphoma ascites tumour cells and Ehrlich ascites tumour cells which are susceptible to the action of Iscador were found to exhibit a material in the sonicates which could inhibit the activity of the isolated component from Iscador (Fig. 1). The inhibitory activity which was quiet pronounced at concentrated sonicates was reversed by diluting the sonictes. The inhibitory activity could be seen only in the case of sonicates from the cells which are susceptible to the action of Iscador and its isolated active component and could not be seen in the sonicates of normal human lymphocytes which were not found to be cytotoxic after incubation with Iscador or its isolated component.

The inhibitor could be seen not only in the cell sonicates of the susceptible cells but also in the ascites fluid (Fig. 2) of Dalton's lymphoma ascites tumour and Ehrlich ascites tumour bearing animals. As in the case of cell sonicates the inhibition was reversed by diluting the ascites fluid prior to incubation with the isolated active component.

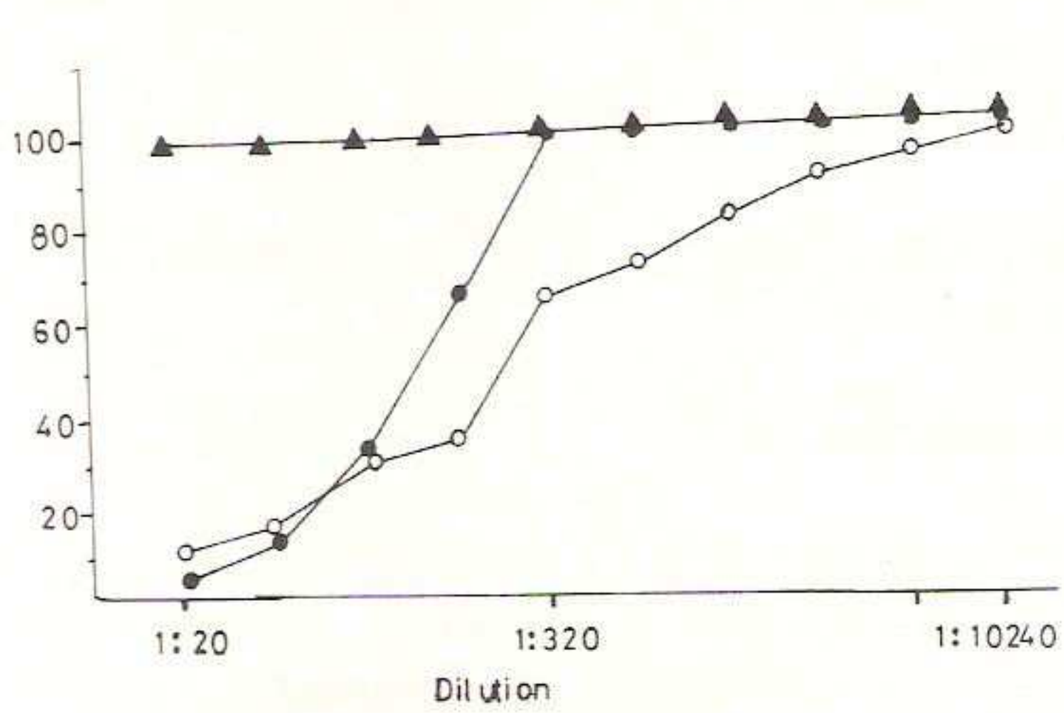

Fig. 1. Inhibition of cytotoxic activity of isolated component from Iscador to Dalton's lymphoma ascites cells with various dilutions of cell sonicates of Dalton's lymphoma cells (●—●), Ehrlich ascites cells (O—O) and normal human lymphocytes cell sonicates (△—△).

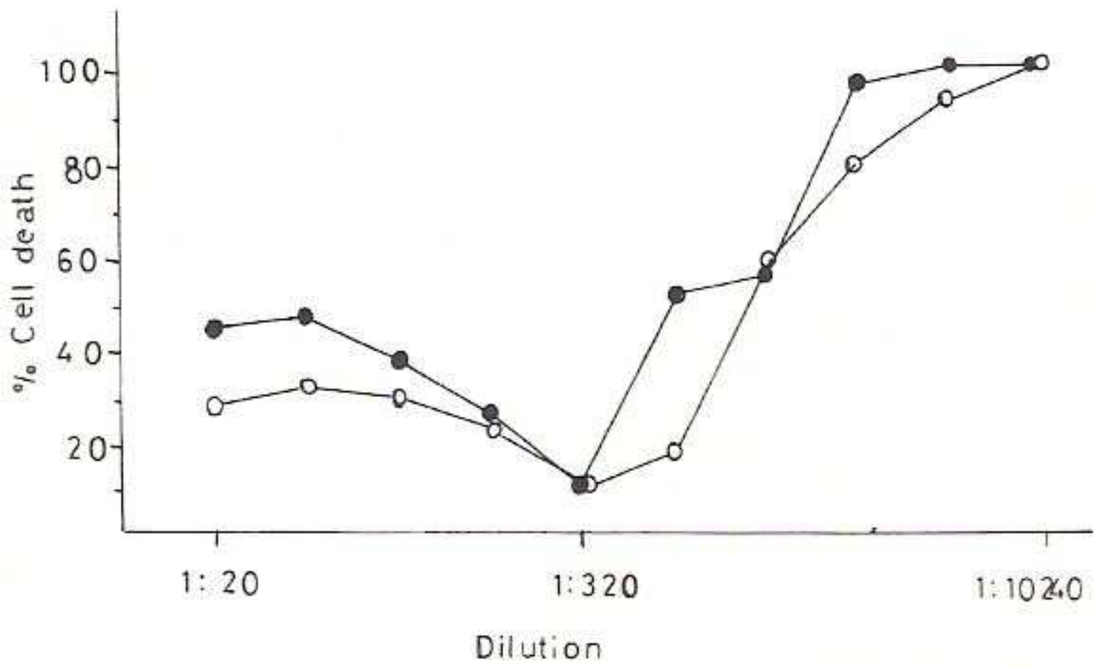

Fig. 2. Inhibition of cytotoxic activity of isolated component from Iscador to Dalton's lymphoma ascites cells (O—O) and Ehrlich ascites cells (●—●) with various dilutions of ascites fluid from tumour bearing animals. Ascites fluid was diluted serially and mixed with isolated component 25 µl (approx. 10 µg) and tumour cells in a total volume of 1 ml and incubated for 3 h at 37 °C. After incubation percentage of live cells was determined by the Trypan Blue exclusion method.

A dose-dependent inhibition by a serially diluted cell sonicate from Dalton's lymphoma ascites tumour cells is shown in Fig. 3. As seen in the figure, the inhibition could be demonstrated in the sonicates incubated with lower concentration of the isolated components (4 µg and 10 µg). When the concentration of the isolated component was higher inhibition could not be demonstrated; indicating either the inhibitory material present may not be of sufficient quantity to inhibit all the activity of the isolated component or due to the presence of other

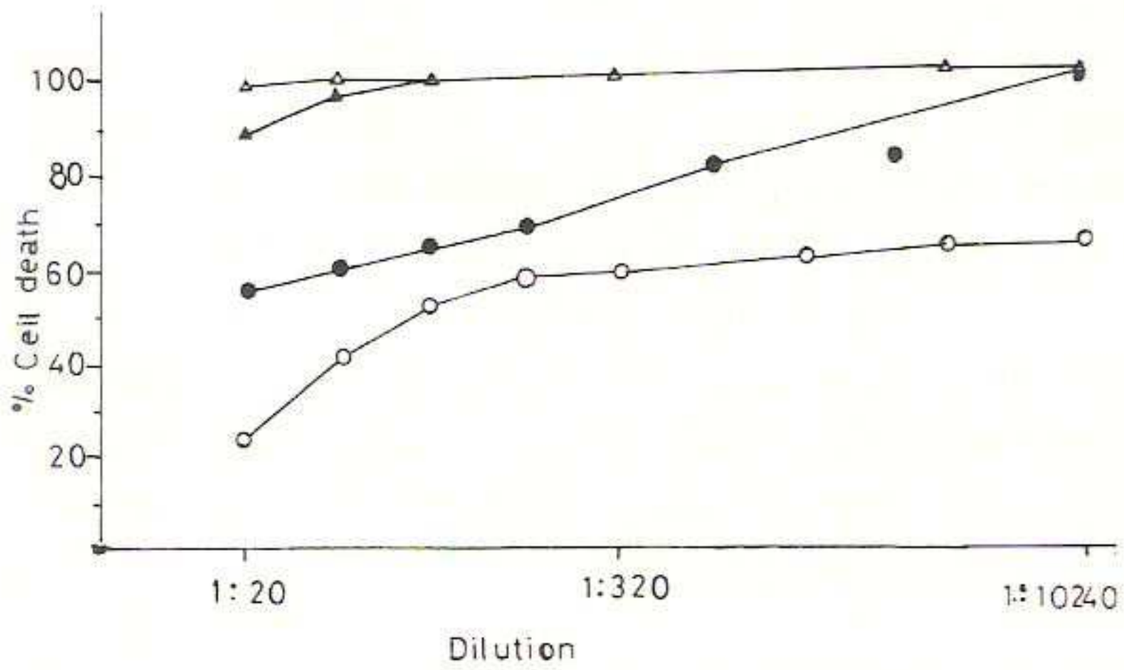

Fig. 3. Dose dependant inhibition of the cytotoxic activity of the violated component from Iscador by serially diluted Dalton's lymphona cell sonicates. Approximate concentration of isolated component used were: O—O, 4 µg; ●—●, 10 µg; △—△, 20 µg; ▲—▲, 40 µg.

cytotoxic components present in the isolated material which are not inhibited by the sonicate.

The inhibitory acivity of the cell sonicates and ascites fluid was destroyed by heat denaturation (60 °C, 10 min) indicating that the inhibitor possibly is a protein. Inhibitory activity could not be seen by addition of bovine serum albumin (10 mg) ruling out the possibility of non-specific protein effect. Similarly, addition of serum also did not produce any inhibition. Dialysis produced a marked loss of activity. However the dialysate did not show any activity which indicated that the loss of activity during dialysis may be due to denaturation.

Sephadex G-50 gel filtration of the ascites fluid indicated that the inhibitory activity was eluted in the void volume of the column indicating a rather high molecular weight (Fig. 4). This also rules out the possible inhibition of cytotoxicity by simple sugars; present in sonicate or ascites fluid which may be possible in the case of lectins reported to be present in Iscador [3].

Discussion

The results presented in this manuscript indicates the presence of an inhibitor for the active component present in Iscador both in the cell sonciates as well as in the ascites fluid of the tumour bearing animals. This inhibitor which is a protein was found to inhibit the cytotoxic activity of Iscador and its isolated active ingredient when incubated in vitro. The inhibitor was not seen in cells which are not susceptible to Iscador. This indicates the possibility that inhibitory protein present in the susceptible cells may be a receptor for the active component present in Iscador and that initial binding of the active component to this receptor is needed to express the cytotoxicity of Iscador and the isolated component. This may also explain the resistance of iscador towards certain human neoplasms and that of animal tumours which have been reported by various authors.

It has been found that the receptor-

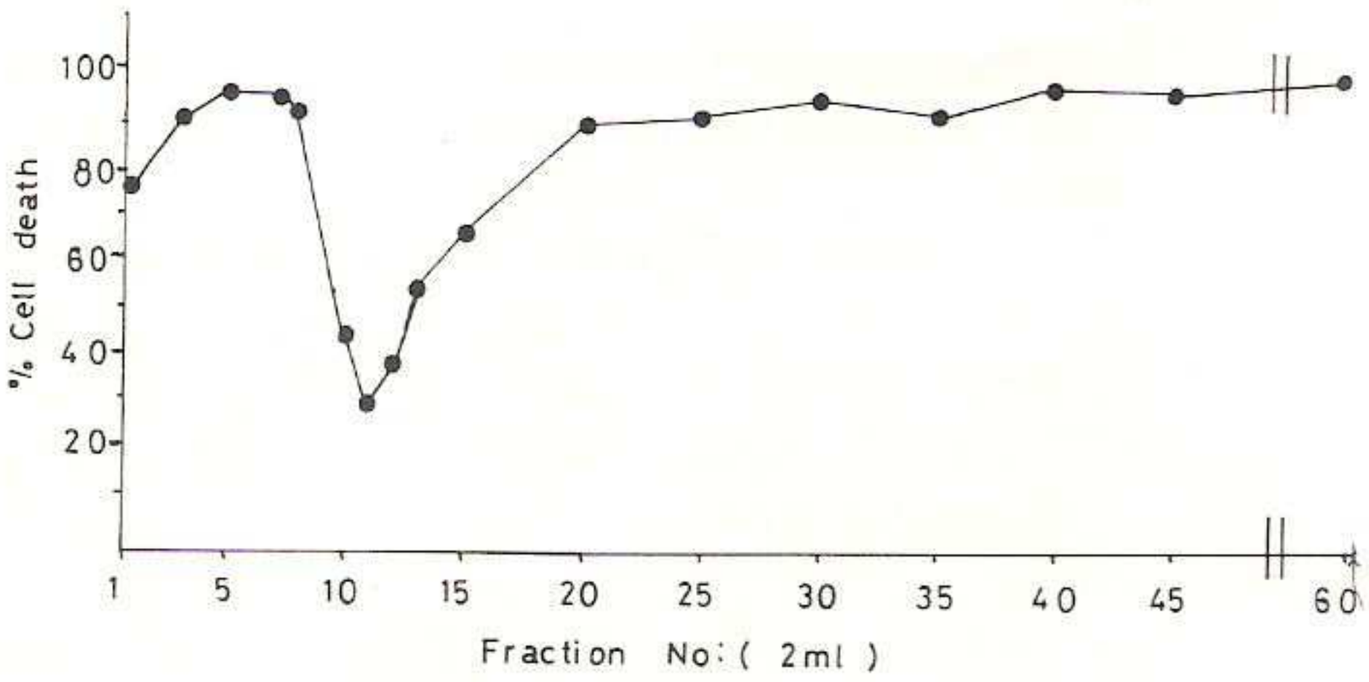

Fig. 4. Sephadex G-50 column chromatography of the receptor present in ascites fluid which inhibited the cytotoxic activity of Iscador. Fractions were assayed for the inhibition of activity as given in the Materials and methods section.

mediated cytotoxicity is the major activity present in Iscdor but a proportion of the cytotoxicity was produced by receptor non-dependent mechanism. Thus, it has always been seen that even with a higher concentration of cell sonicates and ascites fluid only 60—70% cytotoxicity was inhibited whereas 30—40% of the cells were found to be dead. Similarly, growth of Molt. 4 leukemia has been known to be inhibited by non-fermented Iscador [8]. Since Molt. 4 leukemia cells are not susceptible to the action of the component we had isolated (R. Kuttan, unpublished) it is not certain whether this activity is receptor mediated. Moreover we have observed that Iscador as well as isolated component after pronase digestion can produce non-specific cytotoxicity to cells which may not be related to the receptor mechanism.

The inhibition of the cytotoxic activity is not due to the proteolysis of the active component by the ascites fluid or cell sonicates as the inhibition could be seen even after diluting the ascites fluid. Moreover, we had reported earlier that the cytotoxicity of Iscador and its isolated component is not altered by treatment with proteolytic enzymes such as trypsin and pronase [6]. Hence, we assume that the inhibition observed could be due to tightly-binding inhibitor/receptor material present in the ascites fluid and in the cell sonicates.

Exfoliation of a receptor in the ascites fluid of the tumour bearing animals which inhibited the activity of Iscador and its isolated active component is very interesting. Since the exfoliation of antigen and receptors in the ascites fluid is usually regarded as a part of the excape mechanism of tumour cells, the results could indicate that the isolated component from Iscador may be similar or analogous to a body component against which tumour cells produce these receptors to cause inactivation. In fact, it has been observed that Iscador was not effective in treating ascites tumours already developed in animals, obviously due to the presence of a large amount of inhibitory material.

The nature of the active component present in Iscador is not known at present. We reported earlier that the active component is a peptide. However, our recent studies indicate the presence of a small molecular weight cytotoxic component attached to the peptide molecule. The peptide region is attached to a specific receptor on the cells and the cytotoxic component produces cell death. This model explains most of the properties of Iscador and its isolated component.

Acknowledgement

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