Antiproliferative and Anticarcinogenic Effects of an Aqueous Preparation of Abies alba and Viscum album se abies, on a L-1210 Malignant Cell Line and Tumor-Bearing Wistar Rats

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Abstract. Extracts of plants have been widely tested for possible anticarcinogenic properties. In the present study a traditional remedy, consisting of an aqueous extract of mixed parts of the tree Abies alba and its mistletoe Viscum album se abies was tested on benzo(a)pyrene (BaP)-induced tumors in Wistar rats and on the L-1210 malignant cell line. Two main groups of male Wistar rats subcutaneously injected by 10 mg of BaP, a dose inducing 100% carcinogenesis, a control group (C-G, 13 rats) and a treatment group (TR-G, 18 rats), were used for the study. Five animals bearing BaP-induced tumors were also tested (TR-1-G). Animals of the TR-G were orally administered with the aqueous extract at doses of 50 ml/kg b.w. from the day of BaP injection and of the TR-1-G, from the 120th day of injection, till death. L-1210 malignant cells in cultivation, were administered with a powder obtained by condensation and lyophilization of the extract, at various concentrations and cytotoxicity was measured by the microculture tetrazolium assay. Autopsy of the rats, revealed metastasis in the lungs of the animals of all groups and the tumors developed were histologically identified as leiomyosarcomas. The results indicated that the extract of the above plants possess anticarcinogenic effects, documented by: a) its antiproliferative effects on L-1210 cells (IC₅₀ = 49.6 ± 1.4 μg/ml), b) the significant prolongation of life and reduction of tumor growth rate of the animals of the TR-G in comparison to the C-G, c) the inhibition by 16.6% of tumor induction in the TR-G and d) the prolongation of life and the necrotic effects of the extract on the tumors of the animals in the TR-1-G. The antiproliferative effects of the Abies alba and Viscum album se abies extract may be due to the lectins and thionins contained in Viscum album, as well as to the monoterpenes contained in Abies alba. Soft tissue tumors sensitive to the extract, are widespread among human organs, even in larynx, and are usually resistant to chemotherapy.

Various plant and herbal preparations have been used for the differentiation, prevention and treatment of experimental malignancies. Among them substances found in conifers, such as the taxoids, have been established in the treatment of various types of human cancers (1).

Viscum album L. (mistletoe, family Loranthaceae), a plant considered to have various medicinal properties in the folklore of Europe, grows on several species of trees including the apple, oak and fir trees (2).

A preparation of Viscum album L. grown on the apple tree, and known by the name Iscador is reported to be immunostimulating and cytotoxic and has been used for cancer therapy in humans and experimental animals (3-8). Neoplasms reported to respond to Iscador therapy include bladder cancer, breast cancer, larynx cancer, melanoma, ovarian cancer and reticulosarcoma (7,9,10).

There are however tumors and cell lines, such as leukemias, which do not respond to Viscum album preparation treatment (10).

Abies alba is a conifer on which Viscum album se abies grows (11), from which oily and water-alcohol preparations have been studied for their bacteriostatic properties (12,13).

We have previously reported preliminary results of the anticarcinogenic effects of an extract of Abies alba and Viscum album se abies on experimental animals (14).

In the present study an aqueous preparation from both plants Abies alba and Viscum album se abies, that is traditionally administered to cancer patients in various parts of Greece, was tested on the L-1210 malignant cell line and

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tumor-bearing Wistar rats for its antiproliferative and anticarcinogenic effects.

**Materials and Methods**

Different parts of the plant *Abies alba* and its mistletoe, *Viscum album seabies*, were both extracted in tap water by successive boiling and condensations, by a traditional method, described in the patent n. 10002419 (15). The preparations as above were also condensed, dried and lyophilized and powder of the plants' extract was obtained. The lyophilized powder preparation was dispersed in water and tested in malignant cell cultures and male Wistar rats.

**Cell culture tests.** L-1210 cells were cultured in RPMI 1640 medium(Gibco,Life Technologies, Paisley, Scotland, GB) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/ml penicillin, 100µg/ml streptomycin and 10 mM HEPES buffer (pH=7.4). The cells were exposed to graded concentrations of the extract powder (nine serial dilutions in triplicate) for 48 hours. Inhibition of cell proliferation was measured by the microculture tetrazolium assay (16). The results were expressed as IC₅₀, the concentration needed to reduce by 50% the optical density of treated cells with respect to the optical density of untreated controls.

**Animal tests.** Male Wistar rats 3 months old 180±25 g b.w., were subcutaneously injected in the dorsal area with 10.0 mg of benzo(g)pyrene (BP), a dose previously shown to induce 100% tumor development(17,18). The BP-injected rats were then divided into two main groups: a control group(C-G) of 15 animals and a treatment group(TR-G) of 18 animals. The animals of the treatment group were orally admininstered with 50 ml/kg b.w. of the extract, from BP injection till death. The extract as above was also administered to 5 male Wistar rats with well developed tumors(TR-1- G) from the 120th day from BP injection till death.

The animals were followed up till death. Then autopsy was performed, the tumors were carefully excised, weighed and subjected, along with spleen, liver, stomach, pancreas and lungs, to histological examination.

The results were evaluated by the mean survival time and death rate of the animals, the Tumor Growth Rate(TGR), the Carcinogenic Potency of BP (CPBP) and the Anticarcinogenic Potency of the extract(APe), the histological findings and the rate of metastasis in each group.

The TGR was calculated as the quotient of the tumor weight and the survival time of the animal, the CP as the quotient of percentage of tumor induction and the mean survival time of the animals multiplied by 100 in each group and the APe as the difference between the CP of BP in the control group and the CP of BP in the treatment group (17,18) as follows:

\[
\text{Tumor Growth Rate(TGR)} = \frac{\text{Weight of tumor (g)}}{\text{Survival time of the animal (days)}} \quad (\text{g/day})
\]

\[
\text{Carcinogenic Potency of BP(CPBP)} = \frac{\% \text{ of tumor induction}}{\text{Mean survival time (days)}} \times 100 \quad (\text{units})
\]

\[
\text{Anticarcinogenic Potency of the extract (APe)} = \frac{\text{CPBP in C-G} - \text{CPBP in TR-G}}{\text{units}}
\]

The results as above from the treatment group(TR-G) were compared to the control group(C-G) and were statistically evaluated by the Student's unpaired t-test.

**Results**

Treatment of L-1210 cells with the powder extract conferred a 50% inhibition (IC₅₀) at 49.6 (1.4 µg/ml, as estimated by the methods described in Mate- rials and Methods.

All animals of the control group and 15 animals out of 18 of the treatment group, developed tumors at the site of BP injection, between the 80th and 110th day from BP injection, which were histologically identified as leiomyosarcomas.
Table I. Results concerning the administration of the extract on BaP-injected rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean survival time (days)</th>
<th>Tumor growth rate (mg/day)</th>
<th>CPBaP (units)</th>
<th>AP (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C- G (Control)</td>
<td>180 ± 34</td>
<td>1.5 ± 0.6</td>
<td>55</td>
<td>-</td>
</tr>
<tr>
<td>TR-G (Treatment)</td>
<td>240 ± 45*</td>
<td>0.6 ± 0.2**</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td>TR-1-G (Treatment)</td>
<td>222 ± 33</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p<0.001 and **p<0.002 in comparison to control

(Figure 1). In the remaining three animals of the TR-G (16.6%), no tumor development, either on the site of BaP injection or in any of the organs autopsied was identified.

The mean survival time of the TR-G was found to be 240 ± 45 days in contrast to the C-G which was found to be 180 ± 34 days (Table I), resulting in a statistically significant prolongation of life of the animals treated by the extract (p<0.001). The death rate curves of animals in both groups support the beneficial effect of the extract on tumor bearing animals (Figure 2).

The tumor growth rate was also significantly reduced (p<0.002) in the treatment group (0.6 ± 0.2 g/day) in comparison to the control group (1.5 ± 0.6 g/day), as well as the Carcinogenic Potency of BaP (Table I) resulting in an Anticarcinogenic Potency of the extract (APe) of 22 units.

The five animals of the TR-1-G bearing well developed tumors and treated by the extract, survived for 222 ± 33 days and tumors found on autopsy had necrotic cavities in contrast to the solid ones found in the other groups (Figure 3).

Nine of the tumor bearing animals in the C-G (60%) and 10 out of the 15 which developed tumors in the TR-G (66.6%) were found to have single lung metastases, whereas all five animals in the TR-1-G bore lung metastasis.

Discussion

Our data indicated that preparations from the Abies alba and Viscum album se abies, plants growing in the Mediterranean region exert antiproliferative effects on malignant cell cultures and anticarcinogenic effects on BaP-treated animals. These effects were evident from a) the antiproliferative action of the plants preparation, exerted at moderate concentrations, on malignant L-1210 cell lines, b) the prevention of tumor development in 16.6% of BaP-treated rats (TR-G), c) the significant prolongation of life and the reduction of tumor growth rate (TR-G) and d) the necrotic effects of the extract on tumor-bearing Wistar rats (TR-1-G), in comparison to the untreated ones (control group).

Viscum album extracts have been tested for their anticarcinogenic properties in animals and humans in the last decades. Their anticarcinogenic effects are attributed to their cytotoxic and apoptotic activity (19,20,21), their immunostimulant properties (6,7,8) and the antitumor effects on animals and humans (4,7,9,10). Various types of lectins isolated from different species of Viscum album have been considered to possess antiproliferating and apoptotic properties (19,20,22), whereas certain thionins (viscotoxins) along with lectins have shown immunomodulatory and cytotoxic effects (23).

Abies alba is a fir tree found in the Mediterranean region of Europe, on which Viscum album se abies grows. Abies alba extracts have been found to contain monoterpenes and non-monoterpenic fractions through gas chromatography (24,25). Proteins similar to 7S globulins have also been isolated from seeds of Abies alba Taxus bacata and other species of the Pinaceae, Cupressaceae and Taxaceae families (26).

Abies alba oily and water-alcohol preparations exert antimicrobial effects (12,13) but studies dealing with the anticarcinogenic properties of the plant preparations are lacking in the literature. On the other hand, monoterpenes such as those found in Abies alba extracts possess inhibiting and cytotoxic effects on malignant cell lines (27) and on various types of experimental tumor-bearing animals (28,29,30) exerting preventive and therapeutic effects on cancer. This is possibly due to the induction of Phase II carcinogen metabolizing enzymes and the induction of apoptosis through possibly a post-translational isoprenylation of cell growth-regulating proteins (31).

It is well known that plant preparations may exert different effects dependent on the species, the soil in which they grow, the time of harvest and the method of preparation. In the present study an aqueous preparation of mixed parts of the local species Abies alba and Viscum album se abies as well as
their powder, obtained by successive boils and condensations were used. Since chemical analysis of our extract has not yet been performed we can only speculate that substances belonging to monoterpenes and non-terpenes group may exert the anticarcinogenic effects described herewith.

Preparations of aqueous extracts of *Viscum album*, such as Iscador, are also administered subcutaneously, while in our experimental animals the extract was administered orally, thus avoiding local and general adverse effects but probably reducing its efficacy on tumors and/or resulting in the production of unknown metabolites of its active constituents. The effects, however, of its powder, on L-1210 cells, indicated a direct antiproliferative action. It must, to be pointed out that tumors of the soft tissue, such as leiomyosarcomas, are usually resistant to any type of chemotherapy and irradiation, and are found in many organs of humans, even in those of the upper respiratory tract, such as larynx (32). They seem, however, to be sensitive to the extract administered in the present study.

In conclusion, our results on malignant cells and tumor-bearing animals seem promising, calling for further studies on the combined administration of extracts of *Viscum album* along with those of the tree on which it grows, a combination which may have enhanced anticarcinogenic effects.

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**References**


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