Augmentation of Activity of cis-Diamminedichloroplatinum(II) and Mitomycin C by Interferon in Human Malignant Mesothelioma Xenografts in Nude Mice


Department of Neoplastic Diseases, Mount Sinai School of Medicine of the City University of New York, New York, New York 10029

ABSTRACT

Two human mesothelioma xenograft lines, BG and ES, serially passed in athymic mice, were studied to determine the efficacy of α-interferon in this type of tumor. Treatment began after progressive tumor growth was established. Recombinant human α-interferon-2a (Roferon-A) was given by s.c. injection, at a site distant from the tumor, at a dose of 2 x 10^6 IU 5 days per wk for 5 wk. Mild inhibitory activity was noted in both lines with interferon alone. cis-Diamminedichloroplatinum(II) (CDDP) (4 mg/kg) weekly x 5 was effective in line BG, while mitomycin C (1.5 mg/kg) weekly x 3 was effective in line ES. CDDP was not as effective as mitomycin C and CDDP. The combination of mitomycin C and α-interferon was as effective as mitomycin C and CDDP. No additional toxicity was noted by the addition of α-interferon. The combination of recombinant human α-interferon-2a and active chemotherapeutic agents is effective in mesothelioma xenografts.

INTRODUCTION

Malignant mesothelioma, a cancer linked to asbestos exposure, has a uniformly poor prognosis. As a result of the extensive use of asbestos over the past few decades, and because of its delayed oncogenic effect, mesothelioma threatens to become a significant health problem in the future. Epidemiological studies confirm the rising incidence of this disease (1, 2). While few chemotherapeutic agents have exhibited efficacy in treating mesothelioma, activity has been demonstrated in our laboratory using CDDP4 and mitomycin C in nude mouse xenografts (3).

The development of recombinant DNA technology has facilitated research in the field of biological response modifiers. These agents have demonstrated antitumor activity in the treatment of hematological malignancies but have been less successful in solid tumors (4–6). To date, no studies have been reported regarding the use of interferons in the treatment of mesothelioma. An in vitro clonogenic assay performed on the tumor from one of our patients with mesothelioma showed an inhibitory effect by recombinant α-interferon5 at a concentration of 4 ng/ml, a dose that is attainable in vivo (7).

Studies using interferons in combination with conventional chemotherapeutic agents have been carried out in other types of tumors. In this paper we describe the efficacy of rIFN-α-2a alone and in combination with CDDP and mitomycin C in two human mesothelioma xenograft lines.

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2 Present address: Albert Einstein College of Medicine, Dept. of Oncology, 1825 Eastchester Rd., Bronx, NY 10461.
3 To whom requests for reprints should be addressed.
4 The abbreviations used are: CDDP, cis-diamminedichloroplatinum(II); rIFN-α-2a, recombinant human α-interferon-2a; ATTV, average total tumor volume; T/C, final tumor volume of treated arm compared to control arm.
5 The authors thank Hoffmann La Roche, Inc. (Nutley, NJ), for providing the recombinant α-interferon (Roferon).

MATERIALS AND METHODS

Mice. Four-to-6-wk-old female Ncr/nu homozygous (nude) mice were obtained from the Charles River Breeding Laboratory, Inc., through the National Cancer Institute, Bethesda, MD. They were maintained under aseptic conditions which include filtered air and sterilized food, water, bedding, and cages. Experiments began after observing the mice for 2 wk for signs of infection.

Tumor Xenografts. Seven lines of human malignant mesothelioma have been serially transplanted s.c. in our laboratory since 1978, as previously described (8). Their human nature has been confirmed by histological and karyotypical studies (9). Two of these lines (BG and ES) were used for these experiments. Line BG, which is in its sixty-fourth generation, is an epithelial mesothelioma and line ES, used in its seventy-fourth to seventy-ninth generation, is of a mixed epithelial-sarcomatous (predominantly epithelial) cell type.

Transplantation was carried out under sterile conditions under a fiberglass tissue culture hood (Fisher Scientific Co., Pittsburgh, PA). A mouse was sacrificed and its tumors were dissected and trimmed of any adipose or connective tissue. The tumors were then chopped into 1- to 2-mm cubes and using a trocar were placed s.c. into the right (or right and left) axillary area of each mouse. Each mouse received 1 or 2 tumors; all mice in the same study received the same number of tumors.

Treatment Agents. The interferon, provided by Hoffmann La Roche, Inc. (Nutley, NJ), was rIFN-α-2a with a specific activity of 2 x 10^6 IU per mg of protein in the powdered form. The interferon was reconstituted with sterile water and then diluted with sterile normal saline to a concentration of 2 x 10^6 IU/ml. It was given by s.c. injection at a site distant from the tumor. Studies comparing various routes of administration have shown that the s.c. route produces more sustained serum levels of interferon (10).

The chemotherapeutic agents were obtained commercially. The CDDP and mitomycin C (Platinol and Mutamycin; Bristol-Myers Co., Evansville, IN) were reconstituted with sterile normal saline to a concentration of 1 mg/ml and 0.5 mg/ml, respectively. These drugs were administered i.p. on the first day of each wk of treatment simultaneously with the interferon.

Analysis of Activity. The mice were weighed weekly and the tumors measured twice a wk using a caliper. Tumor volumes were calculated using the formula for a prolate ellipsoid

\[ V = \frac{4}{3} \pi LW^2 \]

where \( L \) is the longest diameter and \( W \) the width along the perpendicular axis (11). Measurements were carried out from the first day of treatment to the time the mouse died or its tumor developed necrosis, whichever came first. Two methods were used to evaluate the efficacy of these treatments. The conventional method, described by Geran et al., compares the final tumor volumes in the treated arms to those in the control arm (T/C), with a value of ≤0.42 indicating activity (11).

The other method uses the ATTV which was calculated by taking the area under the curve (i.e., the integral) of the tumor volumes over time for each mouse. This ATTV represents the average height of the volume-time curve throughout the study, thereby giving an overall index of tumor size. A nonparametric analogue of the one-way analysis of variance was used to compare the ATTVs of the mice in all treatment arms to determine overall significance. The Kruskal-Wallis method was used for this analysis because of the small sample sizes. Based upon the determination of overall significance, multiple pair-wise comparisons were then carried out between treatment arms (12).
Treatment. Treatment began 7–12 days after transplantation, after progressive tumor growth was established on two consecutive measurements. The mice were segregated into 4 groups with 4 or 5 mice in each arm of treatment. All of the arms contained the same number of mice and similar sizes of tumors at the start of the experiment. Previous studies in our laboratory indicated that line BG was sensitive to CDDP and line ES sensitive to mitomycin C, and not vice versa, while a combination of the two drugs demonstrated synergy in both lines (3).

Five studies were conducted with one tumor per mouse in four. Study ES74 had two tumors per mouse. The 4 arms in studies BG64 & ES74 were treated as follows: (a) rIFN-α-2a 2 × 10^5 IU s.c. 5 days per wk for 5 wk; (b) CDDP 4 mg/kg i.p. once weekly for 5 wk; (c) a combination of CDDP and α-interferon in the same doses, routes, and schedules; (d) controls, normal saline s.c. in the same volume and schedule as the interferon.

Study ES74 had 4 mice in each arm and utilized mitomycin C, instead of CDDP given at a dose of 1.5 mg/kg i.p. weekly for 3 wk. The interferon was given as above for 5 wk. In study ES78 mitomycin C was again used at the same dose weekly for 3 wk. It was combined with rIFN-α-2a 2 × 10^5 IU administered s.c. 3 times a wk in one arm and 5 times a wk in the other arm.

Finally in study ES79 we compared mitomycin C + α-interferon, versus mitomycin C + CDDP, versus all three agents combined. The interferon in this case was administered for 4 wk, the mitomycin for 3 wk, and the CDDP for 4 wk at the same doses as detailed above.

RESULTS

Figs. 1–4 depict the volumes of the tumors over time for four of the studies. Each point on the curve represents the mean tumor volume for all mice in a treatment arm. Table 1 shows the median ATTVs for each arm after treatment. A, control normal saline; A, rIFN-α-2a 2 × 10^5 IU s.c. 5 days/wk for 5 wk; • CDDP 4 mg/kg i.p. weekly for 5 wk; □, rIFN-α-2a plus CDDP.

Using the conventional method of T/C in BG64, (Fig. 1), rIFN-α-2a alone mildly inhibited tumor growth but not to a significant degree (T/C = 0.70). The activity of CDDP in line BG was reconfirmed with a T/C value of 0.29. When compared to CDDP alone, the combination of rIFN-α-2a and CDDP exhibited markedly enhanced tumor inhibition (T/C = 0.16).

Statistical analysis of ATTVs by pair-wise comparisons yielded significance only for the combination arm versus control (0.005 < P < 0.01). This failure to exhibit significance was felt to be due to the small sample sizes involved in these experiments.

Study ES74 reconfirmed the lack of activity of CDDP in this line and the addition of rIFN-α-2a failed to augment this activity. In ES76 (Fig. 2) rIFN-α-2a inhibited tumor growth to a small extent (T/C = 0.54). Line ES has been shown previously to be sensitive to mitomycin C and again this was confirmed (T/C = 0.33). The addition of rIFN-α-2a improved this activity when compared to mitomycin C alone (T/C = 0.40). Again, we were unable to demonstrate statistical significance in pair-wise comparisons of ATTVs as there were only 4 mice in each treatment arm. Two of the four mice in the combination arm

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<th>Study</th>
<th>Treatment arm compared</th>
<th>Final tumor volume ratio ( T/C )</th>
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<td>BG64</td>
<td>Interferon/control</td>
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<td>CDDP/control</td>
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<td>CDDP + interferon/control</td>
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<td>Mitomycin C/control</td>
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are still alive at 150 days after transplantation without evidence of tumor.

In ES78 (Fig. 3) there was no difference in results when mitomycin C was combined with rIFN-α-2a 3 times weekly as opposed to 5 times weekly at the same daily dose. A comparison in ES79 of mitomycin C and rIFN-α-2a versus mitomycin C and CDDP demonstrated the two regimens to be equally effective. A combination of all three agents could not augment the activity of either of these two already active regimens (Fig. 4).

In all of these studies, there did not appear to be an increase in toxicity brought about by the combination of these two treatment modalities. There were no acute toxic deaths and the weights of the mice were essentially equal in all arms.

**DISCUSSION**

Systemic treatment of many solid tumors, including malignant mesothelioma, is still disappointing. Immunotherapy combined with conventional chemotherapy has been tried in the past with agents such as *Bacillus Calmette-Guérin* and *Corynebacterium parvum*. Results with these agents have for the most part been inconsistent (13). With newer immunological agents, and with the development of recombinant DNA technology, interest has again been aroused in the use of these combined modalities.

In vitro studies using the clonogenic assay system have demonstrated an increase in activity of chemotherapeutic agents by the addition of α-interferon (14, 15). In most cases these effects were additive and in some, synergistic.

Interferons affect cells via a host of mechanisms, both immunological and antiproliferative, and both directly and indirectly (16, 17). The nude mouse model is a useful system to study the direct effects of human interferon on human tumors, as the immunological effects of interferons are species specific (18).

Several studies have been published describing the use of interferons and chemotherapy in the nude mouse xenograft system. Balkwill and Moodie studied recombinant human α-interferon combined with CDDP or cyclophosphamide in human non-small cell lung carcinoma xenografts (19). Carmichael et al. described their experience with interferon and doxorubicin or cyclophosphamide in breast tumor xenografts (20). Both of these studies demonstrated significant augmentation of the cytotoxic effects of the chemotherapy by the addition of α-interferon.

Our studies involved the use of rIFN-α-2a alone and in combination with CDDP or mitomycin C in human mesothelioma xenografts. We were able to demonstrate a marked increase in inhibitory effect with the addition of rIFN-α-2a in BG64 and ES76, respectively.

Heterotransplantation methods allow prediction of clinical results in human tumors (21, 22). According to the methods of Freireich et al., the equivalent human dose of α-interferon was used in these studies, 20 × 10⁶ IU/M², which is a dose at the upper limits of tolerance by patients when given on a daily basis (23, 24). Higher doses have been tolerated when given less frequently (25). Results in our laboratory (ES78) reveal the same efficacy for interferon given 3 times a wk compared to 5 days a wk when combined with mitomycin. Given 3 times weekly, higher doses might be better tolerated in man.

Our last study (ES79) yielded two very interesting findings. The addition of rIFN-α-2a to mitomycin plus CDDP, an already very active regimen, was unable to produce improved results probably because there was little room for improvement. This study needs to be repeated with lower doses of the chemotherapeutic agents. We also showed that the combination of mitomycin and rIFN-α-2a was as active as mitomycin plus CDDP.

Further studies are obviously required to confirm and expand these results. Nevertheless, these studies do suggest that interferon may be useful in certain situations as a substitute or as an enhancing agent for lower doses of standard agents. These results are encouraging and provide a basis for further xenograft studies and for examining whether this might be a valuable therapeutic regimen in patients with malignant mesothelioma and other tumors.

**REFERENCES**


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