Evaluation of the Immunological and Toxicological Properties of MVE-2 in Phase I Trials

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ABSTRACT

Pyran copolymer enhances resistance to infections and transplantable tumors in mice. It induces interferon, activates macrophages, increases antibody-dependent cellular cytotoxicity (ADCC), functions as an adjuvant, and has direct antitumor effects. MVE-2, a low-molecular-weight (15,000) component of pyran copolymer, exhibited less toxicity and essentially the same positive biological effects as pyran copolymer. MVE-2 was, therefore, chosen for clinical trials. This study was designed to determine the toxicity and immunological effects of MVE-2 in humans. Fourteen patients who received biweekly MVE-2 had lymphocyte and monocyte ADCC, natural killer activity, and monocyte to macrophage maturation measured 2, 3, 7, 10, and 13 days after each of the first three doses of MVE-2. Lymphocyte antibody-dependent cellular cytotoxicity and monocyte maturation increased significantly following MVE-2 administration and the effect persisted at least 4 weeks. Although numbers were small, the enhanced ADCC seemed related to both single dose and cumulative dose of MVE-2. Five of six patients receiving more than 2 g of MVE-2 had improvement in lymphocyte ADCC. Increases in lymphocyte and monocyte natural killer activity approached, but did not attain statistical significance. Proteinuria was the dose-limiting toxicity, but was reversible. MVE-2 induced a modest, but real enhancement of lymphocyte and monocyte function at doses that were well tolerated.

INTRODUCTION

Pyran copolymer, the 1,2-copolymer of divinyl ether and maleic anhydride, is a synthetic polyol containing components with molecular weights ranging from 10^2 to 10^7 (2). Pyran copolymer was initially shown in mice to have antiviral effects (4, 8, 20), and subsequently to increase murine resistance to challenge with bacteria, fungi, and protozoa (5, 6, 15, 17). Pyran copolymer pretreatment of mice increased resistance to transplantable tumors (18). This agent is also active in treatment of established tumors in combination with surgery and chemotherapy or as a single agent (5, 14, 19). Specific immune effects include induction of interferon production (11, 13), activation of macrophages (5, 18), and stimulation of ADCC (28). Clinical studies were carried out with a pyran copolymer (NSC 46015; M, 18,000 to 23,000) (21). Toxicities were substantial and discouraged further clinical investigation. The recent discovery that fractions of pyran copolymer with molecular weights from 12,000 to 15,000 retained many of the favorable immunological activities, but had reduced toxicity, stimulated renewed interest in the pyran copolymers (3, 14). The present clinical study of MVE-2 was designed to: (a) define clinical toxicities and determine dose relationships to toxicity; and (b) determine the effect of these doses of MVE-2 on immunological function in humans.

MATERIALS AND METHODS

Patient Population and Study Design. Table 1 presents patient characteristics. Only patients with good functional activity and minimal or no prior immunosuppressive therapy entered the study and were evaluable. Due to delays in drug availability, 2 patients with rapid tumor progression had marked functional impairment, even before treatment. These patients were not subsequently evaluated for alterations in immunological function. MVE-2 was given as a 1-hr i.v. infusion every 2 weeks. Evaluable patients received from 2 to 7 courses of therapy. Blood was drawn for evaluation of lymphocyte and monocyte functions at least once prior to therapy; on Days 2 and 3 posttreatment to evaluate acute immunological effects; and on Days 7, 10, and 13 to evaluate delayed effects. These studies were performed after each of the first 3 doses of MVE-2, with 15 studies scheduled per patient over 6 weeks. In practice, patients had an average of 12.9 complete studies over 45 days, 86% of scheduled studies were obtained. Thereafter, patients received MVE-2 without immunological evaluation. A general immunological evaluation consisting of a battery of skin tests, in vitro lymphocyte blastogenesis to mitogens and antigens, and quantitation of T- and B-cells, was accomplished prior to therapy in all evaluable patients to establish baseline immunocompetence. No patient was anergic and only one had low T- and B-cell numbers. In vitro studies of general immune competence were repeated 2 weeks following the initial dose in 10 of the 14 evaluable patients, and again at 4 weeks in 8 patients for an average of 2 evaluations per patient (range, 1 to 5).

Preparation of Lymphocytes and Monocytes. Plastic adherent and nonadherent cells were isolated as previously described (22, 24). Blood was anticoagulated with EDTA and separated by Ficol-Hypaque gradient centrifugation. The mononuclear interface cells were aspirated and suspended to 4 x 10^6 cells/ml of RPMI with 10% autologous serum. Three ml were then plated on 60-mm culture dishes and incubated for 1 hr. Nonadherent cells were then decanted to a second plate and incubated for an additional 1 hr. The first plate was washed 5 times with Hanks’ balanced salt solution, and residual adherent cells were removed with a lidocaine solution and washed 3 times before utilization in effector cell assays. These cells were greater than 85% monocytes. Cells not adherent to the second plate contained less than 0.5% monocytes and were used as effector cells for the lymphocyte assays.

Lymphocyte Spontaneous Tumoridal Activity (Lymphocyte NK Activity). Molt-4 target cells were removed from 3- to 4-day-old cultures and were labeled with ^51Cr by standard techniques. These targets were suspended to a concentration of 10^6/ml in RPMI supplemented with 10% fetal calf sera. Effector lymphocytes were suspended in the same media at a concentration sufficient to obtain final effector:target ratios of 5:1 and 20:1. One hundred µl of lymphocytes and target cells were added to triplicate 6-mm round-bottomed wells, centrifuged at 200 x g for 5

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with 40% autologous sera was then placed on replicate wells and the cells were incubated for 8 days at 37° in 4% CO₂. Wells were again washed, and the number of surviving adherent macrophages was determined. Data were expressed as the percentage of monocytes which remained adherent at 8 days.

**Interferon Assays.** Interferon was quantitated by its ability to protect a human diploid fibroblast line (MRC-5) from a vesicular stomatitis virus challenge. Serum samples from patients were acidified (pH 2) by the addition of 1.0 N HCl, held for 24 hr at 4°, neutralized with 1.0 N NaOH, and finally clarified by centrifugation. MRC-5 cells were placed in 96-well plastic microtiter trays and allowed to incubate overnight at 37°. Vesicular stomatitis virus (10⁵ plaque-forming units) and serial dilutions of serum samples or a standard reference human interferon obtained from National Institute of Allergy and Infectious Diseases, Resource Reference Reagents, were added to test wells. Cultures were allowed to incubate for an additional 24 hr, at which time the virus control exhibited 100% cytopathic effect; 50% cytopathic effect was used as an end point after staining with crystal violet.

**Lymphocyte Proliferation, T- and B-Cell Numbers and Skin Tests.** In vitro lymphocyte proliferation to phytohemagglutinin, concanavalin A, pokeweed mitogen, and a battery of 4 skin test antigens was measured by [³H]thymidine uptake (9). T- and B-cells were measured by rosetting and immunofluorescence (9). Skin tests were done with phytohemagglutinin, tuberculin, histoplasmin, dermatophytin, Monilia, mumps, and streptokinase-streptodornase (9).

**Statistical analysis.** Analysis of variance was used to evaluate differences between groups, based on immunological parameters. A posteriori comparison of groups deemed significantly different (p < 0.05) following analysis of variance were performed using Scheffe’s test (27).

**RESULTS**

**Toxicity.** The major toxicity of MVE-2 observed in this study was proteinuria that reached nephrotic ranges. Proteinuria was dose related, reversible, and not associated with changes in serum creatinine. Table 2 relates proteinuria to single and cumulative doses of MVE-2. Four of the 11 patients carefully observed for proteinuria developed proteinuria of greater than 1 g/24 hr. Both patients receiving 900 mg/sq m developed substantial but reversible proteinuria. One had a complete clearing of proteinuria over 8 weeks from a maximum value of 3.5 g, despite continued treatment with MVE-2 at a reduced dose of 600 mg/sq m. The second patient, who received 900 mg/sq m, developed proteinuria of greater than 1 g/24 hr at completion of the study. The 7 patients who received MVE-2 at a reduced dose of 600 mg/sq m had adequate follow-up for urine protein after the last dose of MVE-2 and had a proteinuria of less than 1 g/24 hr. Both patients receiving 900 mg/sq m developed proteinuria of greater than 1 g/24 hr at completion of the study. The 7 patients who had adequate follow-up for urine protein after the last dose of MVE-2 and had a proteinuria of less than 1 g/24 hr.

**Partial thromboplastin times and prothrombin times were prolonged after MVE-2 infusion when measured in 8 of 13 patients.** Five patients had from 4 to 8 serial measurements of prothrombin time and partial thromboplastin time immediately following MVE-2. Values showed a maximal increase of 50 to 150% above normal values but were returning to or had reached normal in all patients by 9 hr. Twenty-eight measurements were obtained prior to treatments and after repeated dosings. There were no long-lasting or cumulative effects on either prothrombin time or partial prothrombin time.

Two patients developed mild nausea and/or vomiting. No
Table 2
Proteinuria by dose level

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mg/sq m)</th>
<th>Largest dipsticks*</th>
<th>Maximum 24-hr protein (g)</th>
<th>Courses</th>
<th>Dose (mg/sq m)</th>
<th>Total dose MVE-2 patient received (mg/sq m)</th>
<th>Days to normal</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>Trace</td>
<td>Not measured</td>
<td>3</td>
<td>750</td>
<td>1750</td>
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<td>2</td>
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<td>1600</td>
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<td>800</td>
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<td>600</td>
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<td>2</td>
<td>800</td>
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<td>b</td>
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<td>600</td>
<td>2400</td>
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<td>2400</td>
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<td>1700</td>
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<td>7</td>
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<td>900</td>
<td>Trace</td>
<td>3.47</td>
<td>1</td>
<td>900</td>
<td>3900</td>
<td>28</td>
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</table>

* Dipsticks for proteinuria were done weekly, and 24-hr urine protein was measured before MVE-2 treatment every 2 weeks.

** Urine protein (24 hr) > 50 mg.

Based on 24-hr urine protein measurement obtained every 2 weeks or less if dipsticks are negative. The value is therefore, maximum time to reversal. Four patients were lost to follow-up or died of disease before proteinuria reversed.

* Three low-dose patients were not evaluated for proteinuria.

* Patient not followed to recovery.

Table 3
Values for immunological parameters during therapy with MVE-2

| Parameter | E:T ratio | Base line | First course 0-2 wk | Second course 2-4 wk | Third course >4 wk | p*
|-----------|-----------|-----------|---------------------|---------------------|--------------------|------
| Lymphocyte | Low | 48.1 ± 14.5 | 49.1 ± 17.2 | 58.3 ± 17.0 | 58.7 ± 14.8 | 0.003 |
| ADC | High | 60.5 ± 18.9 | 62.3 ± 13.4 | 67.4 ± 12.7 | 66.1 ± 13.4 | 0.13 |
| Lymphocyte | Low | 48.3 ± 13.3 | 37.0 ± 17.5 | 38.3 ± 21.0 | 42.8 ± 17.6 | 0.23 |
| NK activity | Low | 54.1 ± 11.0 | 42.4 ± 14.8 | 44.6 ± 17.1 | 51.4 ± 20.4 | 0.07 |
| Monocyte | Low | 36.2 ± 15.4 | 27.6 ± 21.0 | 33.9 ± 24.5 | 33.5 ± 23.4 | 0.31 |
| ADC | High | 59.4 ± 21.2 | 44.0 ± 22.5 | 45.8 ± 25.7 | 46.7 ± 24.9 | 0.28 |
| Monocyte | Low | 17.0 ± 16.3 | 27.7 ± 22.0 | 37.6 ± 22.9 | 33.1 ± 23.7 | 0.09 |
| NK activity | High | 64.0 ± 19.4 | 62.0 ± 23.3 | 68.1 ± 19.5 | 57.9 ± 30.0 | 0.34 |
| Monocyte maturation | Low | 45.9 ± 56.2 | 42.4 ± 36.0 | 52.7 ± 37.9 | 85.3 ± 105.8 | 0.04 |

* E:T ratio, ratio of lymphocytes or monocytes to appropriate target cell.

** Analyses of variance between groups.

| Parameter | Mean ± S.D. | Numbers in parentheses, number of studies.
|-----------|-------------|-------------------------------------------------

hypotension, or other clinically apparent adverse side effects were noted. No changes were noted in hepatic enzymes, blood counts, or serum electrolytes.

**Lymphocyte and Monocyte Function.** Table 3 presents sequential changes in lymphocyte and monocyte ADC, NK activity, and monocyte maturation for all patients. No immediate changes in these parameters were noted after any course of MVE-2. All the immunological data are, therefore, summarized in Table 3. Parameters are divided into 4 groups. The first group contains values obtained at base line. The second, third, and fourth groups represent values obtained after the first treatment (Weeks 1 and 2), the second treatment (Weeks 3 and 4), and the third treatment (Weeks 5 and 6). Significant increases in lymphocyte ADC at the lower effector:target ratio and in monocyte maturation were seen. Increases in lymphocyte ADC at the 20:1 ratio, lymphocyte NK activity at the 80:1 ratio, and monocyte NK activity at the 40:1 ratio approached, but did not attain statistical significance. Lymphocyte ADC activity increased within 4 weeks of therapy and then leveled off. These changes occurred gradually. Representative data sets for lymphocyte ADC are shown in Chart 1. Monocyte maturation

![Chart 1](chart1.png)

Chart 1. Sequential effect of MVE-2 on lymphocyte ADC. The values for lymphocyte ADC at a 5:1 ratio are shown. All values for 3 patients (Pt) receiving 600 mg/sq m of MVE-2 and one normal donor (N) are shown. Arrows, treatment. Values for 11 normal individuals were 62 ± 11 (S.D.).

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demonstrated a similar pattern of change. Table 4 relates the single-dose level and the cumulative dose to a statistically significant increase in lymphocyte and monocyte function for each patient. Few patients receiving single doses of less than 400 or 2000 mg/sq m cumulative dose had enhancement of lymphocyte ADCC. Two of the 4 patients receiving the largest cumulative doses did have a significant decrease of monocyte NK activity.

**General Immune Function.** Nine of the 14 evaluable patients had repeated skin tests 2 weeks following therapy, and 6 patients had skin tests repeated at 4 weeks. Six patients developed new reactivity to one of the antigens. This effect may be due to sequential skin testing (16) and is difficult to attribute with certainty to MVE-2. One patient had an abnormally low level of B-cells, and one had a low level of T-cells prior to therapy. Each individual was restested on 4 occasions over the next 5 weeks and failed to attain normal levels. No consistent changes were noted in sequential measurements of T- and B-cell numbers or of lymphoblastic transformation to either mitogens and antigens. Interferon assays were done on 127 occasions in the 14 evaluable patients at 2-week intervals. There was no induction of interferon activity by MVE-2, and all levels remained normal and below 4 IIU/ml.

**Response.** A 35-year-old man had the diagnosis of malignant melanoma, established by biopsy, of a preauricular mass approximately 4 months before receiving MVE-2. During the first month of treatment with MVE-2, a 1-cm preauricular mass which had persisted since surgery disappeared. That patient has remained free of melanoma for 10 months to the time of this writing and represents the only possible response in this study. Three patients had persistence since surgery disappeared. That patient has re

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**Phase I Trials of MVE-2**

<table>
<thead>
<tr>
<th>Dose Level (mg/sq m)</th>
<th>No. of Patients</th>
<th>Lymphocyte ADCC 5:1</th>
<th>Lymphocyte NK activity 20:1</th>
<th>Monocyte ADCC 20:1</th>
<th>Monocyte NK activity 80:1</th>
<th>Monocyte Maturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>4</td>
<td>+</td>
<td>--</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>400</td>
<td>3</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>600</td>
<td>5</td>
<td>+++</td>
<td>+++</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>900</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>0</td>
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</tr>
<tr>
<td>Total dose level</td>
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<td>--</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

*+ or -- indicates patients with a significant increase or significant decrease, respectively, in each in vitro parameter of lymphocyte or monocyte function; 0, no significant changes.*
approached statistical significance ($p = 0.09$).

We were unable to demonstrate induction of interferon production at the doses and schedule of MVE-2 used in this study. This is in contrast to studies showing that pyran copolymer can induce interferon in both animals (13) and humans (11). Similarly, pyran copolymer has been shown to induce suppressor macrophages in mice, which inhibit proliferation of T- and B-cells in vitro (1). We had sequential studies of lymphoblastic transformation in 6 patients, and were unable to demonstrate any consistent and convincing change in transformation.

Renal toxicity is a difficult side effect to deal with, due to the possibility of latent, but clinically very important chronic damage to glomeruli or tubules. This is a particularly important problem when the agent may be used in patients with minimal tumor mass or in an adjuvant setting. This study does suggest that in the absence of proteinuria, intermittent low-dose, long-term therapy can be well tolerated. Animal studies undertaken at Adria Laboratories suggest that rapid infusion of this drug dramatically reduces renal toxicity. We are currently examining this method of administration. Overall, we believe these modest, but potentially important, changes in immune function can be induced by relatively nontoxic dose of MVE-2. This agent deserves further exploration as a biological response modifier.

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