Effects of Methanol Extraction Residue of Bacillus Calmette-Guérin in Humans

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SUMMARY

Forty patients with histologically confirmed neoplastic diseases were treated with the methanol extraction residue of Bacillus Calmette-Guérin (MER). Thirty-six received concomitant chemotherapy. MER was initially given intradermally twice a month, 1 week apart, at a dose of 200 μg into each of five sites draining different lymph node-bearing areas on the anterior body surface. Thirty-seven patients developed local ulcerations at least 0.5 cm in diameter at MER injection sites. Typical lesion evolution was characterized by erythema and induration followed by vesicle formation and central necrosis. Either granulation tissue or a thick nonulcerated eschar preceded healing, leaving a linear, flat scar. Systemic toxicity consisted of malaise, fever, and myalgias on the day of MER administration. No hematological or biochemical changes directly attributable to MER were observed.

Dose titrations in decreasing 10-fold dilutions in a linear array in a single anatomical region were carried out on 35 occasions. All patients but three developed at least a 5-mm induration to the 1-μg dose within 2 weeks of titration. Dose reductions were necessary in 19 instances. The minimal dose that produced a 1-cm inflammatory lesion with central necrosis was 0.01 μg.

Serial biopsies were performed. These indicated a time-related series of changes from a nonspecific inflammatory lesion to an acute inflammatory response with microabcesses, followed by noncaseating granulomata and ultimately fibrosis.

MER is a quantifiable nonviable immunostimulant that obeys dose-response relationships in its cutaneous lesions.

INTRODUCTION

MER,2 the methanol extraction residue of Phipps strain BCG, is a partial purification product of the immunizing components of BCG with the advantages of nonviability and precise dosage. The nonspecific immunostimulant properties of MER were recognized by Weiss in 1956 (9), when guinea pigs that had been immunized with MER to prevent later infection with virulent tubercle bacilli were found during an epidemic to be uniquely resistant to accidental Pasteurella pseudotuberculosis infection. Extensive experiments involving a variety of bacterial pathogens were then carried out. They revealed that MER could elicit some degree of heightened resistance to subsequent challenge with every bacterial pathogen tested (13).

Initial antitumor studies were carried out with MER pretreatment in BALB/c mice injected with an isogenic, spontaneously arising uterine sarcoma. Unlike control animals, no mouse given MER injections developed the sarcoma. Subsequently, pretreatment with MER markedly heightened resistance to various neoplastic isografts, including spontaneously arising and induced neoplasms, both in early and in late transplant generations (12). Increased resistance to the development of leukemia induced by the radiation leukemia virus and to spontaneous breast cancer has also been shown (5, 10).

Under certain experimental conditions, evidence of tumor facilitation was apparent. On the other hand, MER-treated mice also given chemotherapy, therapeutic radiation, or specific tumor immunotherapy never showed facilitation of tumor development. Indeed, MER augmented the retardation, resolution, or cure of the neoplastic process (6, 15).

The mechanism of action of MER is not entirely delineated. Preliminary evidence indicates that it exerts effects directly on cells of the reticuloendothelial system in the development of increased cell-mediated immunity against neoplastic clones (D. W. Weiss, personal communication). Such heightened resistance is not necessarily the outcome of incidental bystander effects or of cross-reactivity between microbacterial and tumor cell antigens (11).

Weiss et al. (14) have demonstrated the efficacy of MER as an immunostimulant and as an agent capable of prolonging remission duration in adult AML. During remission, i.d. MER was given monthly at a dose of 1 to 2 mg into 5 to 10 sites on the back or flanks. In some patients such treatment was given every 2 to 3 months after 15 months of remission. Survival and remission duration for MER-treated patients were statistically significantly prolonged compared to a control group that received maintenance chemotherapy alone (p < 0.05).

Because of the above data, we undertook a study of MER chemoimmunotherapy. The results of this study and of correlates with immunological response to skin tests are presented here.

MATERIALS AND METHODS

Forty patients with histologically confirmed neoplastic diseases have been treated with MER. Patient selection was

1 Supported by Grant 5P01 CA15096-03 from the National Cancer Institute, by the Melvin A. Block Family Foundation, and by Stanley E. Stern.
2 The abbreviations used are: MER, methanol extraction residue of Bacillus Calmette-Guérin; BCG, Bacillus Calmette-Guérin; AML, acute myelocytic leukemia; i.d., intradermal; ESR, erythrocyte sedimentation rate; PPD, purified protein derivative of tuberculin.

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based either upon lack of eligibility for ongoing institutional or group protocols or upon an absence of conventional means of therapy. The study was reviewed and approved by the Human Subjects Committee of the Mount Sinai School of Medicine. Informed consent was obtained from all patients prior to entry. Among 29 patients with breast carcinoma, 11 had Stage II, 7 had Stage III, and 11 had Stage IV disease. There were 3 patients with leiomyosarcoma, 2 with Hodgkin’s disease, 2 with diffuse histiocytic lymphoma, and 1 each with malignant thymoma, osteogenic sarcoma, and melanoma; all had extensive disease. One patient with tongue carcinoma did not. Four patients received MER alone; the remainder received concomitant, intermittent combination chemotherapy. Breast cancer patients received cyclophosphamide, methotrexate, and fluorouracil slightly modified from that used in Ref. 1; some also received vincristine and prednisone in addition (2). Three patients received immunotherapy during a rest period from chemotherapy, while the other 33 received immunotherapy on days when i.v. chemotherapy was given.

MER was supplied by the National Cancer Institute, Bethesda, Md., as a 1-mg/ml sterile suspension. The drug was given with a tuberculin syringe and 25-gauge needle. The vial containing MER and the syringe for injection were frequently agitated to maintain the suspension. MER was initially given i.d. twice each month, 1 week apart, in a dose of 200 μg into each of 5 sites draining different lymph node-bearing areas. In patients with breast carcinoma, regions near the supraclavicular lymph nodes and axilla on the side of the mastectomy were excluded. All injection sites were on the anterior body surface. Cosmetic factors precluded injecting the drug into the upper or lower arm. Acceptable sites for administration included the proximal thighs, upper and lower abdominal wall, lateral thorax, and infraclavicular areas. Measurements of MER ulcerations were made directly. Measurements of induration were made via the ball-point pen method, advancing the point centripetally in perpendicular axes. The diameters of the easily identified borders of induration (where writing stopped) were measured with vernier calipers.

For dose titrations MER was serially diluted by adding 9 ml preservative-free 0.9% NaCl solution to 1 ml of the 1-mg/ml suspension, which was then vigorously agitated. One ml of this solution was transposed to a sterile vial, 9 ml 0.9% NaCl solution were added, and the steps were repeated in a series.

Blood for chemistries and ESR was obtained monthly. A complete blood count was obtained twice monthly in those patients receiving chemoimmunotherapy and monthly in those receiving immunotherapy alone.

Delayed hypersensitivity response to 5 recall antigens was performed at 1- to 3-month intervals. These included PPD (Parke, Davis and Co., Detroit, Mich.); Dermatophyton 0 and Candida (Hollister-Stier Laboratories, Los Angeles, Calif.); streptokinase-streptodornase (Varidase; Lederle Laboratories, Pearl River, N. Y.); and mumps (Eli Lilly & Co., Indianapolis, Ind.). Recall antigens were applied on Day 1 of each treatment cycle to the volar forearm by i.d. inoculation in a 0.1 ml through a 25-gauge needle. Delayed hypersensitivity response to these antigens was read at 48 hr in the same manner as for MER. Skin tests were considered clinically positive if the diameter of induration was 5 mm or greater.

Immunofluorescent studies on skin biopsies were carried out with rabbit anti-lgG, IgA, IgM, the 3rd component of complement, and fibrinogen antibodies.

RESULTS

Thirty-seven patients developed local ulcerations of at least 0.5 cm in diameter at sites of MER injection. In lesions that were to become reactive, erythema and induration developed at the injection site within 48 hr. Shortly thereafter, vesicle formation developed, sometimes as large as 1 cm, followed by central necrosis with a hard maroon-to-black eschar. Ulcerations developed where the eschar had been within 2 months of injection and generally were apparent within 3 weeks. The walls of the ulcer were jagged, and the excavations were one-third to one-half as deep as the diameter. Those patients demonstrating maximal sensitivity to MER had developed ulcers 2 cm in diameter within 1 week of the initial treatment. The ulcers drained a serosanguinous material, which was consistently negative for significant pathogens when tested by gram stain and culture. Some patients tolerated these lesions without complaint, whereas the sores were intolerable to 3 patients. Although the lesions were erythematous and tender, the inflammation was deemed to be due to chemical rather than infectious causes. Lesions healed slowly, either by granulation tissue arising in the base of the ulcer or beneath a thick nonulcerated scab, leaving a linear flat scar that continued to contract over the next 4 to 6 months. Application of antibacterial, steroid, or other ointments was not of benefit in reducing pain or pruritus or in inducing healing of ulcers. In patients with ulcerations 1 cm or more in diameter, local symptoms of moderate severity often persisted for 1 to 3 months. Circumferential rings (bunion pads) comfortably reduced frictional contact with clothing, while direct coverings were often more painful than helpful. Analgesics were not prescribed.

Frequently, lesions from previous injections of MER became reactive with subsequent injections at nearby or remote sites.

No patient developed hepatomegaly or splenomegaly during the course of treatment. Three patients developed lymphadenopathy in a lymph node-bearing area draining MER lesions. In 1 patient with carcinoma of the colon, treated after this study was completed, an ipsilateral inguinal node necrosed and drained following MER injection on the thigh (H. Bruckner, personal communication).

Systemic toxicity commonly consisted of malaise, chilliness, fever, and myalgias on the day of MER administration and shortly thereafter. Five patients reported documented fever of 38.3–39.4° on the day of MER immunotherapy. The frequency of febrile responses did not increase with the duration of treatment. Antipyretic treatment was not used.

No hematological or biochemical changes directly attributable to MER administration were observed. Serial determinations of the ESR were carried out in 22 patients. There was no correlation between the ESR and the severity of the local MER lesions, neither initially nor during the course of treatment.

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Tables 1 to 3 summarize the results of serial skin tests in patients with breast cancer who received chemotherapy or chemotherapy plus MER. No patient with initially negative PPD converted their skin test to positive following MER. Response to Varidase did not increase significantly among patients treated with chemotherapy alone. Among those who were treated with chemoonmunotherapy, significant increase in delayed cutaneous hypersensitivity to Varidase was seen in each stage, although pretreatment values in the various subgroups are variable. Positive responses to Candida increased in each stage after treatment with or without MER.

In an attempt to learn more about human sensitivity to MER and because of the occasional severe local toxicity produced by a 200-μg dose, we carried out titrations on 35 occasions. Decreasing 10-fold dilutions in a linear array in a single anatomical region were carried out once in 13 patients, twice in 4 patients, 3 times in 3 patients, and 5 times in a single patient (Figs. 1 and 2). They were performed at various times during the course of treatment, including the 1st exposure to MER. Increasing sensitivity to MER with serial titrations was demonstrated in 6 patients who were without clinical evidence of disease or who had an objective response to treatment. One patient was less sensitive at the 2nd titration when clinical relapse was evident, and 1, who had increasing and then decreasing sensitivity, ultimately developed clinical signs of relapse. All patients but 3 developed at least 5-mm induration to the 1-μg dose within 2 weeks of titration. Some patients developed >5-mm induration: 1 at a dose of 0.1 μg, 6 at 0.01 μg, 2 at 0.0001 μg (0.1 ng), 1 at 0.000001 μg (1 pg), and 1 at 0.0000001 μg (10−13 g). Dose reductions were necessary in 19 instances because

### Table 1

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<tr>
<th>Recall antigens</th>
<th>Pretherapy</th>
<th>After 5 cycles</th>
<th>p</th>
<th>No. evaluated</th>
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<td>Mumps</td>
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<td>9</td>
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<tr>
<td>Candida</td>
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<td>9.6 ± 3.0</td>
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<td><strong>Chemotherapy + MER</strong></td>
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<td>PPD</td>
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* DCH, delayed cutaneous hypersensitivity.
† Induration ≥ 5 mm.
* Probability that difference between DCH after 5 cycles compared to pretherapy reaction occurred by chance.
* Mean ± S.E.

### Table 2

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<th>Recall antigens</th>
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<th>After 5 cycles</th>
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* DCH, delayed cutaneous hypersensitivity.
† Induration ≥ 5 mm.
* Probability that difference between DCH after 5 cycles compared to pretherapy reaction occurred by chance.
* Mean ± S.E.
of the severity of the local MER response. After dose titrations, the dose arbitrarily adopted was that which led to approximately 1-cm inflammatory lesions with central necrosis as a readily tolerable lesion. Two patients subsequently received a dose of 100 μg in each of 5 sites, 11 received 1 μg, and 3 received a dose of 0.5 μg. Two patients demonstrated inflammatory lesions 1 cm in diameter after only 0.01 μg in each injection site and were thus treated with this dose.

Three patients refused further treatment, 2 at 7 months and 1 at 10 months. Although 4 of the 1st patients had their MER treatment temporarily interrupted to allow healing of ulcers, this procedure is no longer necessary because the adoption of lower doses chosen from the dose titrations prevents the occurrence of excessive multiple ulcerations. Patients are able to tolerate twice monthly injections of MER for at least 12 months with acceptable local, systemic, and emotional toxicity.

Pathology. Biopsies were taken from 26 MER injection sites at various intervals after treatment. Biopsies of skin and underlying s.c. tissue were obtained 3 days to 3 months following injection. The majority were in the 3- to 4-week postinjection period.

The histological appearances suggest a sequential, time-related series of changes. The earliest changes consisted of a diffuse infiltration of the subepidermal tissue with lymphocytes and plasma cells. The epidermis showed no characteristic changes except in the area directly related to the injection site, where a bleb was seen with associated nonspecific inflammatory reaction, including lymphocytes, plasma cells, and occasional neutrophils. In some biopsies, a more brisk neutrophil response was seen, and microabscesses were identified (Fig. 3). Later, typical noncaseating granulomata composed of epitheloid cells, giant cells, lymphocytes, and plasma cells developed. These were focal and scattered through the biopsy specimen (Fig. 4). No granules suggesting particulate foreign material could be identified by light or electron microscopic studies. In the biopsies taken at longer intervals after injection, appearances suggesting resolution were present. Granulomata were present, and lymphocytes, plasma cells, and fibrosis were seen (Fig. 5). In those cases where there was clinically intense redness, edema, and pain, there was an increased frequency of microabscess formation, edema, and hemorrhage; but the basic sequential appearances were essentially similar in all the cases examined.

Immunofluorescent studies were performed on material from 2 biopsies where granulomata were clearly defined by light microscopy. Results were negative for immunoglobulins, complement, and fibrinogen.

In a number of cases, several erythematous areas less than 5 mm in diameter were seen up to 3 cm away from an injection site. These satellite lesions, when biopsied, revealed focal granulomata in the dermis similar to those described above. In 2 patients with AML in remission, participants in a controlled study for that disease (4), satellite lesions too numerous to count developed within a zone approximately 10 cm in diameter surrounding an MER injection site but did not recur with subsequent injections. A lymph node in the drainage area of an injection site became enlarged 11 months after initiation of MER treatment and was biopsied. It also showed noncaseating granulomata essentially similar to those seen in the skin biopsies.

DISCUSSION

MER is an effective immunological stimulant based on both in vivo and in vitro data (5, 11, 12). Its administration on a twice monthly basis is safe and tolerable. Preliminary data (3, 4, 8, 14) indicate clinical efficacy. When given in association with combination chemotherapy in AML (3, 4, 14) and neuroblastoma (8), significant increases in response duration and survival have been reported when compared to patients treated with chemotherapy alone. Likewise, it has been reported to induce a low order of
responses when used alone in advanced gastrointestinal carcinoma. Moertel et al. (7) treated 40 patients with advanced gastrointestinal cancer with MER. They administered 2 mg injected i.d. in divided doses into 5 sites on the back, either on weekly or 4-weekly schedules. Among 36 patients with measurable disease, all chemotherapy failures, 3 showed greater than a 50% objective response.

Unlike BCG, MER is a product of a nonviable mycobacterial fraction. Thus, it cannot cause systemic infection. Furthermore, MER appears distinct from that BCG fraction that stimulates reactivity to PPD since our patients did not convert to positive PPD responses. MER also has the advantage of precise dosing, which permits extensive studies of dose parameters and comparability of patient data.

REFERENCES

Fig. 3. MER microabscess. H & E, × 400.
Fig. 4. MER granuloma. H & E, × 400.
Fig. 5. MER resolving lesion. H & E, × 100.
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