Immunotherapy of L1210 Leukemia Using Neuraminidase-modified Plasma Membranes Combined with Chemotherapy

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ABSTRACT

Purified L1210 plasma membranes treated with Vibrio cholerae neuraminidase (VCN) were used for active immunotherapy of L1210 tumors in DBA/2J mice. Immunotherapy with VCN-treated membranes was effective only when combined with 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU). Successful therapy was a function of the dose of MeCCNU, the dose of VCN-treated membranes, and the time after MeCCNU treatment when VCN-treated membranes were administered. Optimum conditions for treating animals with tumors initiated with 10^6 cells were MeCCNU (20 mg/kg) given 3 days after tumor inoculation and 0.25 mg VCN-treated membranes given 1 day after chemotherapy. Control membranes, not treated with VCN, that were administered 1 day after MeCCNU were ineffective; when given 4 days after chemotherapy, they caused accelerated mortality, suggesting immunological enhancement of tumor growth. Our results indicate that VCN-treated plasma membranes can be used for active immunotherapy of established tumors and underscore the importance of carefully designing immunotherapy protocols to achieve optimum desirable effects.

INTRODUCTION

VCN2-treated, nondoning, viable tumor cells have been used for active immunotherapy of established tumors in animals and humans. Administration of nondoning, VCN-treated, methylcholanthrene-induced fibrosarcoma cells to tumor-bearing animals slows the growth of the tumor (28, 29) and in some animals causes it to completely regress (27). VCN-treated tumor cells also induce regression of spontaneous canine mammary tumors (25), B16 melanoma (18), L1210 leukemia (2, 3), and murine pulmonary (1) and squamous cell (16) carcinomas. VCN-treated tumor cells have been used to treat residual tumors in patients with mammary carcinomas, gastrointestinal tumors, skin cancers (20), and bronchogenic carcinomas (30) after the main tumor masses have been surgically removed. They have also been combined with chemotherapy to treat patients with acute myelogenous leukemia (2, 3). The disease-free interval in treated patients was longer than that in control patients in all of the above cases.

Immunotherapy with VCN-treated tumors is effective only when tumors are small (17) or when the tumor burden has been reduced by surgery (17) or chemotherapy. Viable VCN-treated cells are not essential for therapy; enzyme-treated, frozen-and-thawed cells are equally effective (13, 18). These observations suggest that VCN-treated, purified tumor cell membranes could be effective vaccines for active immunotherapy of small tumors or residual tumors after surgery or chemotherapy.

In the previous paper (4), we demonstrated that treatment of DBA/2J mice with purified, VCN-treated L1210 plasma membranes protected them against a supralethal challenge of L1210 cells. We described the preparation of modified plasma membranes and demonstrated that the only modification was the removal of NANA. In this report, we describe the treatment of established L1210 tumors with VCN-treated L1210 plasma membranes combined with MeCCNU chemotherapy.

MATERIALS AND METHODS

Animals. Female DBA/2J mice weighing approximately 20 g were purchased from The Jackson Laboratory (Bar Harbor, Maine).

L1210 Tumor. The L1210 tumor line used (LE50B01) was obtained from the National Cancer Institute tumor bank through Arthur D. Little Co., Inc. (Cambridge, Mass.). The tumor was maintained by weekly i.p. passage of 10^5 cells in DBA/2J mice.

Plasma Membrane Preparation. Plasma membranes were prepared from L1210 ascites tumors as described in the previous paper (4).

VCN Treatment of Plasma Membranes. The conditions for treating L1210 plasma membranes with VCN to remove sialic acid from glycoprotein and glycolipid oligosaccharide side chains are described in the previous paper (4). Control (untreated) membranes were incubated under the same conditions without VCN.

Chemotherapy. MeCCNU was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. It was stored at -85°. Suspensions of the drug were prepared fresh before each use by warming it to room temperature in a desiccator, rapidly weighing the amount required, and sonicating it in 0.8% NaCl solution-0.05% Tween 80 for 30 to 60 sec. Mice receiving chemotherapy were given i.p. injections of 0.5-ml suspensions of the drug.

Immunotherapy. We used a protocol similar to that of Sanding and Kollmorgen (23) for immunotherapy without chemotherapy. Sixty female DBA/2J mice were each inoculated with 100 L1210 cells and randomly divided into 3 groups of 20. Five days after tumor inoculation, one group received i.p. injections of 0.2 mg of VCN-treated membranes suspended in 0.1 ml of HBSS, a second group received untreated membranes suspended in 0.1 ml of HBSS, and the third group received 0.1 ml of HBSS only and served as the no-treatment control group.
For chemoimmunotherapy experiments, 80 mice were each given i.p. injections of 10⁴ L1210 cells. Sixty of these were treated with MeCCNU as described above; the remaining 20 received i.p. injections of 0.8% NaCl solution-0.05% Tween 80 and served as the no-treatment control group. Those receiving chemotherapy were randomly divided into 3 groups of 20. The mice in each group received either VCN-treated membranes suspended in 0.1 ml of HBSS, untreated membranes suspended in 0.1 ml of HBSS, or 0.1 ml of HBSS only (chemotherapy control). The doses of MeCCNU or membranes and the time after tumor inoculation when the drug or membranes were administered were varied.

In both the immunotherapy and the chemoimmunotherapy experiments, the animals in the experimental and control groups were observed daily, and mortality was recorded as a function of time after tumor inoculation.

RESULTS

Immunotherapy without Chemotherapy. We used conditions similar to those of Sansing and Kollmorgen (23) to determine whether i.p. injections of VCN-treated or untreated L1210 plasma membranes induced a remission of established L1210 tumors. The amount of untreated membrane protein administered (0.2 mg) was chosen so that the amount of NANA that it contained would be the same as that in 1.5 x 10⁷ L1210 cells (23); 0.2 mg of VCN-treated membranes had that amount of NANA removed from them.

The median survival time of mice receiving no treatment was 16 days; this value agrees with that reported by Sansing and Kollmorgen (23). Administration of either VCN-treated or untreated membranes did not prolong survival or induce regression of tumors. Therefore, using the conditions reported by Sansing and Kollmorgen (23), we found that VCN-treated or untreated L1210 membrane preparations did not induce regression of established tumors in DBA/2J mice.

Chemotherapy of L1210 Tumor-bearing Mice with MeCCNU. Bekesi et al. (2) and Le Fever et al. (13) found that immunotherapy with VCN-treated, nondividing (2), or frozen-and-thawed cells (13) was effective only if mice were first given chemotherapy. In their experiments, chemotherapy increased the median survival time of tumor-bearing mice, which was further increased by the use of VCN-treated cells.

We were unable to reproduce the results obtained by either of these groups with chemotherapy only. Using their conditions of initial dose of tumor, dose of drug, and the interval between tumor inoculation and drug administration, we found that the median survival time of treated mice was the same as that of control mice. This suggested that, in our hands, the chemotherapy protocols of Bekesi et al. (2) and Le Fever et al. (13) did not reduce the tumor burden. Therefore, we performed chemotherapy trials with MeCCNU to find conditions that would have the desired effect when MeCCNU was combined with treatment with VCNTreated and untreated membranes. The desired effect was defined as prolongation of the median survival time of treated mice without curing a high percentage. Under these conditions, a positive effect of additional therapy (VCN-treated or untreated tumor cell membranes) would manifest itself as further prolongation of survival.

We performed chemotherapy trials in DBA/2J mice bearing ascites tumors initiated with 10⁴ L1210 cells. MeCCNU was administered at different times after tumor inoculation, mice were observed daily, and deaths were recorded as they occurred. As illustrated in Chart 1, 60% of mice receiving MeCCNU (20 mg/kg) 1 day after tumor inoculation were cured. Because of this high percentage of cures, these conditions of chemotherapy are unsuitable for our experiments. No additional improvement was noted in these animals when chemotherapy was combined with immunotherapy using VCN-treated L1210 plasma membranes (data not shown). In contrast, when MeCCNU was administered at a dose of 20 mg/kg 3 days after tumor inoculation or at a dose of 10 mg/kg 3, 4, or 6.5 days after tumor inoculation, the median survival time of treated mice was 8, 6, and 4 days longer than that of untreated mice. In addition, a large percentage of mice were not cured. We used all of these protocols for combination therapy with MeCCNU and VCN-treated and untreated L1210 plasma membranes.

Combination Therapy of L1210 Tumor-bearing Mice with MeCCNU and VCN-treated or Untreated L1210 Plasma Membranes. Chart 2 illustrates the results of an experiment in which L1210 ascites tumor-bearing mice were given MeCCNU (10 mg/kg) on Day 4 after inoculation of 10⁴ L1210 cells and VCN-treated or untreated L1210 plasma membranes (0.2 mg/mouse) on Day 6. Chemotherapy improved the median survival time. However, combining MeCCNU with VCN-treated or untreated membranes did not result in additional prolongation of survival. Similar results (data not shown) were obtained when MeCCNU was administered on Day 3 and the membranes were given on Day 6 or when the drug was administered on Day 6.5 and the membranes were given on Day 8. These results could be due to an insufficient dose of MeCCNU, or plasma membranes could be ineffective. To distinguish among these possibilities, we performed combination therapy using a higher dose of MeCCNU.

Chart 3 illustrates the results of a combination therapy experiment in which the dose of MeCCNU was increased to 20 mg/kg. In this experiment, the drug was administered on Day 3, and VCN-treated or untreated plasma membranes (0.2 mg) were administered on Day 6. The median survival time of mice receiving the MeCCNU and VCN-treated membranes was 5 days longer than that of mice receiving chemotherapy only. This demonstrates that VCN-modified L1210 plasma membranes are effective when used in combined therapy. The failure of experiments described above and illustrated in Chart 2 was apparently due to an insufficient dose of MeCCNU.
Chart 3 also illustrates the results of combination therapy using MeCCNU and untreated L1210 plasma membranes. Mice receiving MeCCNU and untreated membranes died sooner than did those receiving MeCCNU only. Thus, under the conditions of this experiment, untreated membranes enhanced tumor growth.

We also varied the dose of plasma membranes used in combined therapy. In these experiments, MeCCNU (20 mg/kg) was administered on Day 3, and membranes were administered on Day 4. The results of these experiments are illustrated in Chart 4. Unlike the experiment illustrated in Chart 3, untreated membranes at doses of 0.25 mg (Chart 4A), 0.125 mg (Chart 4B), or 0.025 mg (Chart 4C), when given 1 day after chemotherapy, did not enhance tumor growth. The median survival time of these mice was the same as that of mice receiving chemotherapy only. However, the percentage of mice receiving chemotherapy and 0.25 or 0.125 mg of untreated L1210 plasma membranes that survived longer than 60 days was greater than that of mice treated with chemotherapy only.

VCN-treated membranes were more effective than untreated membranes. The most effective dose of VCN-treated membranes administered 1 day after chemotherapy was 0.25 mg (Chart 4A); the median survival time of this group was 17 days longer than that of the group receiving chemotherapy only. Because this protocol was more effective than that used for the experiment illustrated in Chart 3, it suggests that the timing of therapy is important. Moreover, because lower doses of VCN-treated membranes (Chart 4, B and C) were only marginally effective, there may be a threshold dose for effective therapy.

DISCUSSION

In the preceding paper (4), we demonstrated that VCN treatment of purified L1210 plasma membranes enhanced their...
therapeutic potential. We showed that the most probable reason for this is exposure of galactose and N-acetylgalactosamine termini on plasma membrane glycoprotein oligosaccharides. Moreover, the modified membranes protected DBA/2J mice against challenge by viable L1210 cells. The present study demonstrates that these preparations can also be used to treat actively growing tumors.

Sansing and Kollmorgen (23) reported complete remission of tumors in 80% of mice given injections of VCN-treated, nonviable L1210 cells 5 days after i.p. inoculation of viable L1210 cells. We used VCN-treated L1210 cell plasma membranes under similar conditions and found VCN-treated L1210 plasma membranes inhibited the growth of established L1210 tumors in DBA/2J mice only when used in conjunction with MeCCNU chemotherapy. This is consistent with the findings of others that VCN-treated cells are effective only against small tumors or after the tumor burden has been reduced (17). However, because the VCN-treated membranes were ineffective without chemotherapy when the tumor was initiated with as few as 100 L1210 cells, chemotherapy may be needed for reasons other than reduction of tumor burden. For example, in the reports of Kilion and Kollmorgen (11) and Kilion (10), it was shown that subpopulations of L1210 cells differ in their immunogenicity, and Mihich and Kitano (15) demonstrated that L1210 tumor cells that survive chemotherapy are more immunogenic than is the untreated tumor. Therefore, chemotherapy may eliminate less immunogenic cells; hence, those that survive this treatment are more susceptible to therapy with modified plasma membranes.

Conditions for successful combination therapy of growing L1210 tumors with MeCCNU and VCN-treated membranes were arrived at empirically. The doses of drug and membranes and the time after tumor inoculation when the drug and membranes are administered are important variables. Untreated membranes either enhanced tumor growth or were less effective than VCN-treated membranes, demonstrating the importance of VCN modification in maximizing the therapeutic potential of L1210 plasma membranes. Factors such as tumor burden, changes in the cell population of a growing tumor, and the immune status of a tumor-bearing host may also influence the outcome of this type of therapy. Additional work is needed to define all parameters that affect successful therapy with VCN-treated plasma membranes; this would be of aid in developing protocols that are maximally beneficial in treating growing tumors.

Our conditions for successful chemoimmunotherapy of L1210 leukemia with VCN-treated plasma membranes differ from those of Bekesi et al. (2, 3), who used VCN-treated, nondividing L1210 cells combined with MeCCNU chemotherapy, and from those of Le Fever et al. (13), who used frozen-and-thawed, VCN-treated L1210 cells combined with 1,3-bis(2-chloroethyl)-1-nitrosourea chemotherapy. One explanation for this is that these investigators used different inbred strains of mice in their experiments than we used in ours. Bekesi et al. (2, 3) used DBA/2Ha mice, a subline of DBA/2 mice different than the one in which the L1210 tumor originated (12). Moreover, DBA/2Ha mice immune to L1210 cells show accelerated rejection of DBA/2J skin, suggesting that there is an antigenic incompatibility between the L1210 tumor and the DBA/2Ha line (14). Similarly, Le Fever et al. (13) used C57BL/6 x DBA/2 F1 (hereafter called BD2F1) hybrids in their experiments. L1210 tumor cells grown in these mice have a longer doubling time than do L1210 cells grown in DBA/2J mice (6). This phenomenon has been ascribed to allogeneic inhibition of tumor growth in BD2F1 mice (6) and suggests that there is also an antigenic incompatibility between the L1210 tumor and the BD2F1 host.

The tumor-host incompatibility in the experimental systems of Bekesi et al. (2, 3) and Le Fever et al. (13) could influence the outcome of their experiments as well as the optimal protocol for successful therapy and may account for our inability to reproduce their results. In contrast to their experimental systems, our experiments were performed using DBA/2J mice, the same subline in which the tumor originated (12); therefore, the outcome of our experiments is not influenced by a tumor-host incompatibility.

VCN treatment may enhance the therapeutic potential of plasma membranes by one of several mechanisms. The enzyme may increase the content of carbohydrate-associated antigens (5, 7, 9, 22) by modifying oligosaccharide side chains so that immunization with modified cells or membranes could enhance the immune response to antigens on the cell surface that are not normally present in large enough amounts to elicit a strong immune response. Alternatively, cytotoxins to VCN-treated cells that are found in many normal sera (8, 19, 21, 26) may enhance the binding of enzymatically treated cells in peritoneal macrophages (14, 24), resulting in macrophages that are cytotoxic (26). Additional experiments are needed to distinguish which of these mechanisms is more important in tumor immunity.

Vaccines prepared from purified plasma membranes have an advantage over those prepared from whole cells in that fewer extraneous contaminants are introduced into the host. In addition, because they are more stable than whole cells, they should be more applicable for clinical medicine. Moreover, because they are a subcellular component the composition of which can be defined, they should provide an improved system over whole cells for analyzing the modifications resulting from VCN treatment and relating these to biological activity.

REFERENCES


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