Combination Chemotherapy with 1-(4-Amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea Hydrochloride and Bleomycin in Meningeal Carcinomatosis in Rats

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ABSTRACT

Combination chemotherapy with 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU) and bleomycin (BLM) was evaluated using an experimental model of meningeal carcinomatosis induced in Sprague-Dawley rats by intracisternal inoculation of $1 \times 10^6$ Walker 256 tumor cells.

Tumor-bearing animals were treated by i.v. administration of cyclophosphamide, BLM, ACNU, or a combination thereof, starting on Day 5 after tumor inoculation. BLM, 5 mg/kg on Day 5 as well as 5 mg/kg/day on Days 5, 7, 9, 11, and 13, was ineffective. Cyclophosphamide, 30 mg/kg, or ACNU, 15 or 30 mg/kg, on Day 5 increased the median survival time by 52, 70, and 82%, respectively. The combination of ACNU, 15 mg/kg, and cyclophosphamide, 15 mg/kg, increased median survival time by 73%, while the combination of ACNU, 15 mg/kg, and BLM, 5 mg/kg, resulted in a maximal increase of median survival time of 200% when the agents were given on Day 5. The combination of ACNU, 15 mg/kg on Day 5, and BLM, 5 mg/kg/day on Days 5, 7, 9, 11, and 13, increased median survival time by over 360% and cured 60% of the animals.

These results point to the therapeutic advantage inherent in ACNU and BLM combination therapy.

INTRODUCTION

To date, no single oncolytic agent has been proved sufficiently effective against human neoplasms. Efforts have been directed to the use of multiple drug combinations in order to increase tumor cell kill.

We tested the efficacy of combined ACNU3-BLM therapy, using an experimental model of meningeal carcinomatosis induced in rats by i.c. inoculation of Walker 256 tumor cells (12). This regimen was based on the observation that ACNU accumulates tumor cells in the G2-M phase (9) and that BLM is most actively potent on G2-M-phase cells (2).

MATERIALS AND METHODS

Details regarding the present meningeal carcinomatosis model have been described previously (12). Walker 256 carcinosarcoma was obtained from the Research Institute of Microbial Diseases, Osaka University, and is being maintained by serial s.c. transplantation in our laboratory. Nine days after s.c. transplantation, the tumor was removed aseptically, minced with fine scissors in Earle's basic medium, and passed first through 40 mesh and then through 80 mesh stainless steel screens under aseptic conditions. Viable cells ($1 \times 10^6/0.1$ ml of Earle's medium) were obtained using the trypsin blue exclusion test for viability.

Female Sprague-Dawley rats weighing approximately 150 g were anesthetized i.p. with sodium pentobarbital, 20 mg/kg, and $1 \times 10^4$ viable tumor cells were injected p.c. into the cisterna magna, using a No. 26 needle.

On the fifth postinoculation day, the rats were divided randomly into groups of 10 rats each, and groups were treated by different regimens. In each experiment, one group served as a nontreated control.

Single-Agent Chemotherapy. BLM, supplied by Nihon Kayaku Co., Tokyo, Japan, in vials containing 15 mg BLM, was dissolved in 7.5 ml sterile water and injected i.v. via the tail vein in a single 5-mg/kg dose on Day 5 or in 5-mg/kg doses on Days 5, 7, 9, 11, and 13 after tumor inoculation.

ACNU, supplied by Sankyo Co., Tokyo, Japan, in vials containing 50 mg ACNU, was dissolved in sterile water to obtain 6 mg ACNU per ml water. Animals were treated with a single i.v. dose of 15 or 30 mg/kg on Day 5 after tumor inoculation.

Cyclophosphamide, supplied by Shionogi and Co., Ltd., Osaka, Japan, in vials containing 500 mg, was dissolved in 42 ml sterile water and injected i.v. via the tail vein as a single 30-mg/kg dose on Day 5 after tumor inoculation.

Combination Regimen. Three combinations were tested: ACNU, 15 mg/kg, plus cyclophosphamide, 15 mg/kg i.v. on Day 5; ACNU, 15 mg/kg, plus BLM, 5 mg/kg i.v., on Day 5; and ACNU, 15 mg/kg i.v. on Day 5, plus BLM, 5 mg/kg i.v. on Days 5, 7, 9, 11, and 13 after tumor inoculation.

All animals were checked daily, and the day of death was recorded for each rat. The life spans of treated rats were compared to those of untreated animals and analyzed by the Wilcoxon rank sum test. Long-term survival was determined to a maximum of 60 days after tumor inoculation. In selected experiments, body weight was recorded every other day. Autopsies were performed on several animals from each group, and histopathological examination was performed as described previously (12).

RESULTS

Most of the animals died within 7 to 13 days, and all of the
untreated rats died by Day 20. The histopathological findings were similar to those previously reported and consisted of diffuse or multifocal tumor invasion of the leptomeninges (12).

Table 1 summarizes the results of single-agent chemotherapy. In each group, the median day of death of the treated rats was compared to that of the control rats, and the median life span was determined as the percentage of the control. While cyclophosphamide significantly prolonged the survival time by 52%, BLM in either single or multiple doses was not effective. ACNU, 15 and 30 mg/kg, increased the survival time by 70 and 82%, respectively.

Table 2 summarizes the results of combination chemotherapy. Compared to the other combinations tested, ACNU and cyclophosphamide caused no significant prolongation of survival time. The combined therapy of ACNU, 15 mg/kg, and cyclophosphamide caused no significant prolongation of survival time. The combined therapy of ACNU, 15 mg/kg, and cyclophosphamide caused no significant prolongation of survival time. The combined therapy of ACNU, 15 mg/kg, and cyclophosphamide caused no significant prolongation of survival time. The combined therapy of ACNU, 15 mg/kg, and cyclophosphamide caused no significant prolongation of survival time. The combined therapy of ACNU, 15 mg/kg, and cyclophosphamide caused no significant prolongation of survival time.

Table 2 shows that the effect of BLM plus ACNU was synergistic and that the survival time of tumor-bearing animals treated with this combination was significantly prolonged as compared to the other treatment regimens investigated. Because the side effects of ACNU and BLM are entirely different, toxicity as judged by weight loss was not greater in this combination than in single-agent chemotherapy.

DISCUSSION

BLM is one of the ideal agents for use in combination with other antineoplastic agents because it does not possess myelosuppressive or immunosuppressive activities (1, 6). Several trials have been made to combine BLM with vincristine, methotrexate, or 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (4, 5, 7, 8, 10, 11). Livingston et al. (4) treated cancer patients with vincristine and BLM in sequential combination in an attempt to accumulate tumor cells in mitosis with vincristine (3) for subsequent destruction by BLM (2). This attempt revealed some evidence of the efficacy of this combination therapy. However, because their study as well as others was not controlled, the superiority of the combined treatment was not proven.

Recent in vivo pulse-cytophotometric studies of Shitara et al. (9) revealed that ACNU causes tumor cells to accumulate in the G2-M phase. Therefore, we tested the efficacy of combined chemotherapy with BLM and ACNU using an experimental model of meningeal carcinomatosis. Our present model imitates the clinical situation, and it is thought that a treatment study using this model can be used to predict the clinical therapeutic response (13).

Our results clearly demonstrated that the effect of BLM plus ACNU is synergistic and that the survival time of tumor-bearing animals treated with this combination was significantly prolonged as compared to the other treatment regimens investigated. Because the side effects of ACNU and BLM are entirely different, toxicity as judged by weight loss was not greater in this combination than in single-agent chemotherapy.

These findings suggest that combination chemotherapy with BLM and ACNU appears promising and warrants clinical trials.
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 although the exact mechanisms for the synergistic effects of this combination are not clear and are a subject to be investigated.

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REFERENCES

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