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Therapy of patients with metastatic breast cancer with 5-fluorouracil, leucovorin and carboplatin

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Thirty-four women with metastatic breast cancer were treated at the National Cancer Institute of the National Institutes of Health, with a regimen of leucovorin (L), 500 mg/m^2 i.v. over 30 min, followed in 1 h by 5-fluorouracil (5-FU), 375 mg/m^2 i.v. bolus on days 1–5, and carboplatin (CBDCA), 50–100 mg/m^2 i.v. bolus on days 2–4, every 28 days. All patients had received previous combination chemotherapy with at least one regimen (29 patients with 5-FU-containing regimens). CBDCA, 100 mg/m^2 on days 2–4, resulted in grade 4 neutropenia in 10 out of 11 patients associated with sepsis in all 10 patients. CBDCA, 75 mg/m^2 (seven patients) and 50 mg/m^2 (15 patients), resulted in grade 4 neutropenia in six and eight patients, and neutropenic sepsis in five and two cases, respectively. Grade 4 thrombocytopenia occurred in 10, five and two patients receiving 100, 75 and 50 mg/m^2 of CBDCA, respectively. Other toxicities included grade 3/4 mucositis in 18 patients and grade 3/4 diarrhea in 10 patients. Twenty nine patients were evaluable for response, with one pathologic complete response (3%), two partial responses (6%), 18 stable disease (53%) and eight (24%) progressive disease. Sites of response included bone, viscera and soft tissue. The median time from entry on study to progression, for responders, was 15 months. When platinum–DNA adduct formation in peripheral white blood cells was analyzed in 27 patients at 24 h after drug administration, a significant correlation between adduct level and CBDCA cumulative dose was found. However, no statistically significant correlation was found between platinum–DNA adduct formation and disease response, survival or toxicity in this study. The results presented here suggest that the combination of 5-FU, L and CBDCA is tolerable at a CBDCA dose of 50 mg/m^2 days 2–4; however, higher doses of CBDCA are poorly tolerated due to hematologic toxicity. At the CBDCA dose level of 50 mg/m^2 on days 2–4, no significant disease responses were observed. We conclude that higher doses of CBDCA are highly toxic and limited our ability to deliver adequate doses of L-modulated 5-FU.

Key words: Advanced breast cancer, carboplatin, DNA addict, 5-fluorouracil, leucovorin.

Introduction

Patients with metastatic carcinoma of the breast who have failed standard chemotherapeutic programs have an overall poor prognosis. Although several combination chemotherapy regimens result in relatively high response rates, the median response duration and survival is generally less than 1 year.[1,2] It is clear that the development of new therapeutic strategies is needed for this patient population. Previous studies have demonstrated that the combination of 5-fluorouracil (5-FU) and 5-formyl-tetrahydrofolate [leucovorin (L), citrovorum factor] is active in the salvage treatment of patients with metastatic breast cancer.[3–5] A substantial body of experimental pre-clinical and clinical data[6–12] has indicated that the addition of pharmacologic concentrations of reduced folates to human tumor cells *in vitro* and patients enhances both the duration and degree of thymidylate synthase inhibition produced by 5-FU. There is also an observed synergy between 5-FU and cisplatin. In

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animal models and tissue culture systems the mechanism of this interaction is unclear; however, studies in murine models suggest that the sequence of 5-FU preceding platinum may be important.[13,14] The combination of 5-FU and cisplatin has been applied to the treatment of a variety of solid tumors, including carcinoma of the head and neck,[15–18] gastrointestinal cancer,[19] non-small cell lung cancer,[20] breast cancer and colorectal carcinoma.[21–23] In several of these reports the combination was found to yield results that were greater than would be expected from a simple additive effect of the two agents.

This study was established based on the promising results of the combination of 5-FU and L as salvage therapy in patients with breast cancer and the data indicating a synergistic effect of 5-FU and cisplatin. Carboplatin (CBDCA) is a cyclobutane dicarboxylate second generation derivative of cisplatin. It was chosen for inclusion in this investigation since the dose-limiting toxicities of cisplatin, such as neurotoxicity and ototoxicity, limit its use in a prolonged treatment regimen, as is needed in patients with breast cancer.

Previous investigations have shown that the extent of platinum–DNA adduct formation measured in the peripheral mononuclear cell DNA of ovarian and testicular cancer patients treated with cisplatin or CBCDA may be associated with disease response.[24–27] In this study we investigated platinum–DNA adduct formation in the peripheral blood mononuclear cells of breast cancer patients receiving CBDCA, 5-FU and L in order to assess any association with dose, toxicity, disease response or survival in breast cancer patients.

Materials and methods

Eligibility

To be eligible for the study, patients were required to have histologically documented, measurable metastatic breast carcinoma; adequate bone marrow reserve (WBC $\geq$ 4000 cells/ml) and platelets ($\geq$100 000/ml), unless due to bone marrow involvement by tumor; creatinine clearance >60 ml/min; serum SGOT <50 IU/l; serum bilirubin <1.5 mg/dl; and prothrombin time <14.0 s. All patients gave informed consent according to the guidelines of the Institutional Review Board of the National Cancer Institute. Patients may have had prior chemotherapy.

Pre-treatment evaluation and follow-up studies

Pre-clinical evaluation included a complete history and physical examination, complete blood count, screening biochemistry profile, urinalysis, bone scan, metastatic bone survey, chest X-ray, mammogram, liver–spleen scan and photographs of visible lesions. Computed tomography and bone marrow examinations were performed as warranted by clinical judgment. All patients had weekly CBC counts. Patients had a biochemistry profile, chest X-ray, measurement of evaluable disease, photographs of all patients with skin disease, physical examination and interval history every 4 weeks. All patients had a bone scan, liver–spleen scan (if previously abnormal), chest X-ray and X-ray of sites of known bone disease every 3 months.

Study design

The chemotherapy consisted of 5-FU, L and CBDCA. L, 500 mg/m^2, was administered i.v. over 30 min, followed in 1 h by 5-FU, 375 mg/m^2 i.v. bolus on days 1–5, and CBDCA, 50–100 mg/m^2, was administered i.v. bolus from days 2 to 4. The treatment was repeated every 28 days. The dose of CBDCA was reduced by 25 mg/m^2/day (on days 2–4) for the next cycle in patients with nadir WBC <1000 or platelets <25 000. If after dose reduction of CBDCA to 50 mg/m^2/day the blood counts were still unacceptable (nadir WBC <1000 or platelets <25000; day 1 WBC $\leq$3000 or platelets <75 000; or a 1 week delay) then the dose of 5-FU was decreased to 300 mg/m^2 day.

WBC–DNA adduct levels

Peripheral blood samples from 29 patients were obtained 24 h following the completion of day 4 CBDCA. Whole blood was centrifuged to obtain the buffy coat (nucleated blood cells) and DNA was isolated as previously described.[26–28] CBDCA–DNA adduct formation was determined by an enzyme linked immunosorbent assay (ELISA), using a polyclonal antiserum elicited against cisplatin-modified calf thymus DNA. Blood samples from patients who received a total dose of 600 mg/m^2 (between two and four cycles) were used to reduce the bias in the length of follow-up for those patients with progressive disease. Platinum–DNA adduct levels were analyzed for an association with the cumula-

tive dose of CBDCA, disease response, survival and toxicity.

Criteria for response

Complete response (CR) was defined as the disappearance of all objective evidence of disease on two separate measurements at least 4 weeks apart. Partial response (PR) was defined as a decrease of 50% or more in the sum of the products of the diameter of the measurable lesion(s), without evidence of new lesions for two consecutive evaluations separated by at least 4 weeks. The same criteria were used whether single or multiple lesions were evaluated. Progressive disease (PD) was defined as an increase of 25% or more in the area of the measurable lesion(s) or the appearance of new lesions. Patients with tumors not meeting these criteria for response or progression were considered stable (SD).

Statistical methods

Survival proportions were computed using the method of Kaplan and Meier.[29] Associations between survival time and other variables were assessed by means of the likelihood ratio test of the Cox proportional hazards model. Spearman rank correlations were calculated for platinum–DNA adduct levels, CBDCA dose and toxicity. The Wilcoxon rank sum test was used for the associations of adduct level and dose with tumor response. All p values reported are two-tailed.

Results

Of the 34 consecutive patients that were entered into this study, 29 patients were evaluable for response. Five patients were considered not evaluable for disease response; two experienced early toxic death and three patients were lost to follow up 1–2 months after the initiation of therapy. The two toxic deaths occurred 7 and 10 days after the first course of chemotherapy. In the first patient, the platelet count at the time of death was found to be less than 10 000/mm^3; death occurred away from our center and an autopsy was not performed. The second patient died at home 10 days after the first cycle of chemotherapy. The WBC count was less than 1000/mm^3 1 day prior to the event; however, the patient reported no signs or symptoms suggestive of infection and was afebrile. An autopsy revealed no apparent cause of death, and postmortem blood, urine and cerebrospinal fluid cultures were negative. The characteristics of all patients are shown in Table 1. All patients had received previous combination chemotherapy with 29 (85%) having received and progressed on previous therapy with 5-FU-containing regimens.

Toxicities

The combination of 5-FU, L and CBDCA at the highest dose (100 mg/m^2) was associated with severe toxicity (Table 2). The most severe toxicity was hematologic, 10 out of 11 patients (91%) who received CBDCA at 100 mg/m^2 had nadir WBC counts $\leq$2000 and platelets counts $\leq$10 000. Two patients died 7 and 10 days after the first course of chemotherapy, as described above. All patients required 25–50% dose reduction of CBDCA after the

Table 1. Patient characteristics

Total number of patients		34
evaluable for response		29
evaluable for toxicity		34
Median age in years (range)		50 (31–67)
Median Karnofsky performance status (range)		80 (30–90)
Estrogen receptor status of primary tumor		
positive		14 (41%)
negative		14 (41%)
unknown		6 (18%)
Menopausal status at the time of diagnosis		
pre-menopausal		20 (58%)
post-menopausal		13 (38%)
unknown		1 (3%)
Prior therapy		
prior 5-FU		29 (85%)
time since last dose of 5-FU	$<$1 year	14 (41%)
	$>$1 year	10 (59%)
number of previous chemotherapy regimen		
	1	23 (67%)
	$\geq$2	11 (32%)
hormonal therapy	1	21 (61%)
	$>$2	9 (35%)
radiotherapy	yes	4 (12%)
	no	30 (88%)
immunotoxin therapy		1 (3%)
Sites of disease		
bone		20 (59%)
visceral		18 (53%)
soft tissue		13 (24%)
others		2 (6%)
Number of sites of disease		
1		14 (41%)
2		15 (44%)
3		5 (14%)

Table 2. Toxicity of 5-FU, L and CBDCA

Toxicity	Grade	Dose of CBDCA (mg/m^2) (no. of patients)		
		100 (11)	75 (7)	50 (16)
Hematologic				
WBC	2	1 (9%)	0	4 (25%)
	3	0	1 (14%)	4 (25%)
	4	10 (91%)	6 (86%)	8 (50%)
platelet	≤2	1 (9%)	1 (14%)	9 (56%)
	3	0	1 (14%)	5 (31%)
	4	10 (91%)	5 (71%)	2 (13%)
Gastrointestinal				
liver toxicity	1	3 (9%)		
stomatitis	≤2	7 (64%)	1 (14%)	8 (50%)
	3	2 (18%)	4 (57%)	4 (25%)
	4	2 (18%)	2 (29%)	4 (24%)
diarrhea	≤2	9 (82%)	2 (29%)	13 (81%)
	3	1 (9%)	5 (71%)	3 (19%)
	4	1 (9%)	0	0

first cycle and therapy was delayed for up to 7 weeks after the first cycle. At a dose level of 75 mg/m^2, six out of seven patients had grade 4 neutropenia and five out of seven patients had grade 4 thrombocytopenia. The lowest dose tested (50 mg/m^2) was better tolerated, eight out of 16 patients had grade 4 neutropenia and two out of 16 patients (13%) had thrombocytopenia. Nine patients (31%) required 5-FU dose reduction to 300 mg/m^2 due to gastrointestinal toxicity. The most severe gastrointestinal toxicity was stomatitis, which occurred in nine patients.

Response

Among 29 patients evaluable for disease response, CR was observed in one patient who had skin disease. PR was seen in two patients (6%) and 18 patients (53%) had SD. Eight patients (24%) progressed after two cycles of therapy. The objective response rate for all patients was 9%.

Survival

The time to progression for the PRs was 13 and 14 months. One patient who had skin involvement only had a CR and remains disease free after 50 months. Among the patients with SD, the median time to progression was 9 months from the date of entry into the study. The median survival for all patients was 11 months (Figure 1).

Patients with a Karnofsky performance status of 90 or higher had significantly longer survival times than patients with lower performance status ($p^2 < 0.01$). However, no associations between survival and age, disease stage, estrogen receptor (ER) status, menopausal status, or prior therapy were found.

Correlation of CBDCA–DNA adducts with CBDCA dose, disease response, survival and toxicity

Blood from 29 patients was obtained 24 h following the completion of day 4 CBDCA. These samples were analyzed for CBDCA–DNA adduct formation in the peripheral mononuclear cells by ELISA. Only 27 patients were evaluable for disease response, from whom DNA adducts were measured. The average number of samples assayed per patient was 4.2 (range 1–10). There was a high level of intrapatient variability in the platinum–DNA adduct levels of the samples. For example, one patient had a level over 2000 attomol/mg DNA during cycle 1 and no detectable adduct formation during cycle 2.

In order to obtain a single value that summarized the repeated samples from each patient, we calculated the average of the DNA adduct levels. Since adduct formation is cumulative, we also determined the cumulative CBDCA doses at which the samples were drawn and calculated the average of those doses. The average adduct level and the average

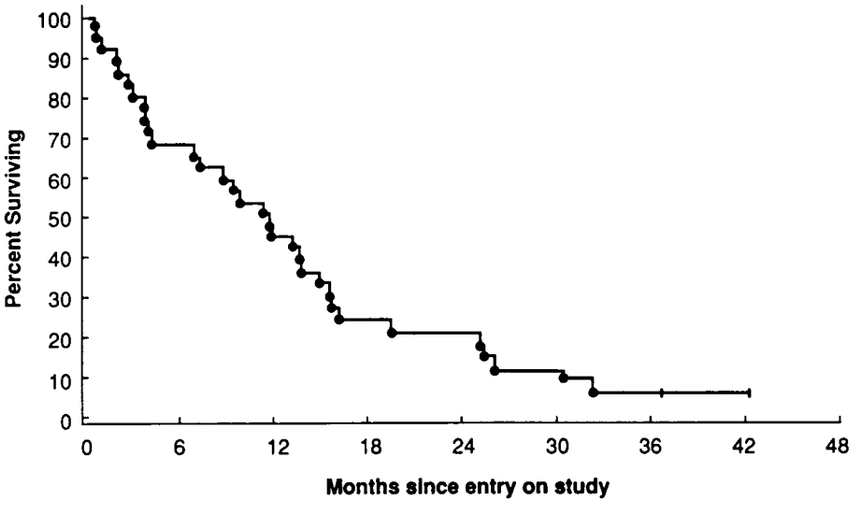

Figure 1. The Kaplan–Meier survival curve for all 34 patients entered in the study. Survival is calculated from the time of entry into the study. The median survival is 11 months. (●) All patients (32/34) failed.

dose are significantly correlated ($r = 0.53$, $p^2 < 0.005$ for the null hypothesis of zero correlation).

The number of patients whose tumors responded to therapy was too small to test for association with average adduct level. Therefore, we compared adduct levels in six patients with PD against the levels in 20 patients with SD or tumor response. The PD patients remained on therapy for at most three cycles and thus received lower cumulative doses than most of the other patients. In contrast, 13 of the non-PD patients received four or more cycles of therapy. To reduce this bias caused by the length of therapy, we analyzed only samples drawn at cumulative CBDCA doses up to and including 600 mg/m^2. The median adduct levels for the group of six patients experiencing PD was 2.75 (range 0–226.9) attomol/mg DNA, and for the 19 patients experiencing either CR, PR or SD was 30.9 (range 0–2606.6) attomol/mg DNA. However, neither adduct level nor dose is significantly associated with PD in this subset of the data ($p^2 = 0.48$ and 0.65, respectively). However, in the cross-tabulation shown in Table 3, the correlation between adduct level and dose was still significant ($r = 0.45$, $p^2 < 0.025$).

There was no apparent association between the average adduct level and either the highest grade of toxicity or the survival time of the patients. Toxicity grade was marginally related to cumulative dose ($r = 0.40$, $p^2 < 0.05$).

Table 3. Numbers of patients in categories formed by average CBDCA cumulative dose and average DNA adduct level ($p = 0.007$ by Mantel–Haenszel test for trend in row and column ranks)

Average of adduct levels (attomol/mg DNA)	Average of cumulative CBDCA doses (mg/m^2)		
	<300	300–350	350–600
0	4	5	2
1–100	3	4	4
>100	0	0	6

Conclusion

The present report suggests that the combination of 5-FU, L and CBDCA in previously treated patients with metastatic breast cancer is poorly tolerated. A CBDCA dose of 50 mg/m^2 on days 2–4 could be given with acceptable toxicity; however, higher doses of CBDCA caused sever hematologic toxicity. The combination of 5-FU and high-dose L in previously treated breast cancer patients has been shown to induce an objective response in 20–30% of the patients.[3–5] In our trial, objective response was seen in only three patients (9%) with 18 patients (53%) maintaining SD. This low response rate may be attributed to the high frequency of dose reduction (all patients required dose reduction, 25–50% of the initial dose of CBDCA) and

1–3 weeks dose delay due to severe hematologic toxicity. In addition, 85% of our patients had received previous therapy with 5-FU. The poor tolerance of this combination regimen in our trial may, in part, be attributed to the fact that all patients had received previous combination chemotherapy. Abbruzzese *et al.*[30] have shown in a phase I clinical trial of cisplatin given i.v. day 1 with 5-FU 375 mg/m^2 days 1–5 and leucovorin 500 mg/m^2 days 1–5 that the maximum tolerated dose of cisplatin was 75 mg/m^2. The dose-limiting toxic effect of this regimen was myelosuppression, as was the case in the present trial. O'Dwyer *et al.*[31] treated 26 patients with varying doses of 5-FU by continuous infusion for 5 days; 200 mg/m^2 L by daily bolus injection for 5 days and 20 mg/m^2 cisplatin also for 5 days. These investigators demonstrated that patients with poor performance status and extensive prior radiation tolerated the regimen poorly. The maximum tolerated dose of 5-FU in this group was 200 mg/m^2 daily. Patients with good performance status and no prior chemotherapy tolerated 300 mg/m^2 of 5-FU.

The median survival in this trial, 11 months, is similar to the previous published experience with 5-FU and L,[3] where the median survival was 8 months.

In this study, we also examined CBDCA–DNA adduct formation in peripheral mononuclear cells from 27 patients. We found a significant correlation between adduct level and CBDCA cumulative dose in patients grouped by adduct level. Individuals with the poorest clinical responses were observed to have lower median DNA adduct levels than the other patients. In contrast to previously reported studies in testicular and ovarian cancer patients,[25,27] the difference reported here was not statistically significant even though it was in the same direction. We also found that adduct levels did not correlate with survival or toxicity level. It is possible that this difference is due to either the low response rate observed in this study or the relative insensitivity of breast cancer to platinum and platinum analogs compared with ovarian and testicular cancer.

We conclude that in previously treated breast cancer patients, the combination of 5-FU, L and CBDCA is a relatively toxic regimen. A dose of 50 mg/m^2 CBDCA could be given with acceptable toxicity. At this dose level, no significant disease responses were observed. Although the combination of these three agents has been shown to be highly effective in the treatment of head and neck cancer, this study did not demonstrate that CBDCA increased the activity of 5-FU and L.

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