Continuous Infusion High-Dose Leucovorin with 5-Fluorouracil and Cisplatin for Untreated Stage IV Carcinoma of the Head and Neck

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Study Objective: To study the activity of continuous infusion cisplatin, 5-fluorouracil, and high-dose leucovorin (PFL) as induction chemotherapy in patients with previously untreated, advanced squamous cell carcinoma of the head and neck.

Design: Nonrandomized, prospective trial.

Setting: A comprehensive cancer center.

Patients: Thirty-five patients (4 patients [11%], stage III; 31 patients [89%], stage IV [M0]), all evaluable for response and toxicity.

Interventions: Two to three cycles of PFL before definitive, local-regional therapy (surgery and radiation therapy or radiation therapy alone). Chemotherapy included continuous intravenous infusion of cisplatin (25 mg/m^2 body surface area daily, days 1 through 5); 5-fluorouracil (800 mg/m^2 body surface area daily, days 2 through 6); and leucovorin (500 mg/m^2 body surface area daily, days 1 through 6) administered once every 28 days. Pathologic response was evaluated by surgical resection or biopsy. Serum-reduced folates were measured before and 18 hours after the initiation of chemotherapy.

Results: A clinical response to PFL was achieved in 28 of 35 (80%) patients: 23 (66%) patients had a complete response (90% CI, 50% to 79%) and 5 (14%) patients, a partial response. A complete response was confirmed pathologically in 14 of 19 (74%) patients. The most common toxicity was mucositis (grade 2 to 3; 94% of patients). Dose reduction for toxicity was necessary in 11 (31%) patients. There were no treatment-related deaths. Serum levels of leucovorin and (6S)5-methyltetrahydrofolate were measured in 7 patients. After 18 hours, the mean leucovorin level ($\pm$ SD) was 34.3 $\pm$ 1.5 μmol/L, of which only 8.0 $\pm$ 0.5% was the active 6S isomer. The mean serum (6S)5-methyltetrahydrofolate was 9.2 $\pm$ 0.6 μmol/L.

Conclusions: Continuous infusion cisplatin, 5-fluorouracil, and high-dose leucovorin is a new and highly active chemotherapy regimen that can achieve clinical and pathologically confirmed complete responses in a substantial proportion of patients with advanced, local-regional squamous cell carcinoma of the head and neck. Further studies are needed to confirm the activity of PFL and to determine its potential impact on local tumor control and disease-free and overall survival.

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During the past decade many randomized trials (1-7) have studied the value of induction combination chemotherapy for patients with advanced squamous cell carcinoma of the head and neck. Although a survival advantage has not been shown, there was a significant correlation between response and survival in these as well as in other studies (8-12). It has been postulated that the potential benefit of induction chemotherapy will remain difficult to document until regimens are available that consistently produce a complete response rate exceeding 50% (13). The initial study (14) of cisplatin and 5-fluorouracil (PF), probably the most widely used combination chemotherapy for squamous cell carcinoma of the head and neck, suggested that a complete response rate of 54% can be achieved with this regimen. Continued evaluation in patients with advanced stage III to IV (M0) squamous cell carcinoma of the head and neck indicated, however, that the true complete response rate after three cycles of PF is in fact lower, between 20% and 35% with three cycles of therapy (15).

To improve the activity of PF, many different treatment strategies have been studied, including multiple courses (more than three cycles) of therapy (16, 17), cisplatin in high doses (18, 19), the substitution of carboplatin for cisplatin (20), and the addition of other cytotoxic agents to the regimen, such as methotrexate and bleomycin (16, 17, 21). However, to date these approaches have either failed to produce a significant improvement in the complete response rate or have been associated with prohibitive toxicities. It has been shown both in vitro and in vivo that the cytotoxicity of 5-fluorouracil is increased by leucovorin (22-26), which can be attributed to more effective inhibition of the enzyme thymidylate synthase. Based on this observation and the potential advantages of the administration of cisplatin and 5-fluorouracil by continuous infusion (27-31), a new regimen (PFL) was devised in which high-dose leucovorin was added to a modified PF regimen. In PFL, all three drugs are administered intravenously by continuous infusion, with cisplatin and leucovorin started 24 hours before 5-fluorouracil. The objective of this phase II study was the clinical and pharmacologic evaluation of PFL as induction therapy for patients with previously untreated squamous cell carcinoma of the head and neck.

Materials and Methods

Patient Characteristics and Treatment

Between July 1987 and May 1989, 37 patients with previously untreated, advanced squamous cell carcinoma of the head

and neck entered a Dana-Farber Cancer Institute protocol of induction therapy with cisplatin, 5-fluorouracil, and leucovorin (PFL) followed by conventional therapy (surgery and radiation therapy or radiation therapy alone). The first 35 patients to complete PFL therapy are the subject of this report. Eligibility was limited to patients with stage III and IV (M0) disease, excluding patients with T3N0 exophytic and T1N1 tumors. Patients were required to have bidimensionally measurable, pathologically confirmed squamous cell carcinoma of the head and neck; a performance status of 0 to 2 (Eastern Cooperative Oncology Group classification); an adequate enteral diet; adequate bone marrow reserve (leukocyte count $> 4 \times 10^9$/L [4000 cells/μL]; platelet count $> 10 \times 10^9$/L [100 000 cells/μL]); normal aspartate aminotransferase (AST) and bilirubin levels; a creatinine level $\leq$ 110 μmol/L [1.2 mg/dL]; and a 24-hour creatinine clearance $\geq$ 0.83 mL/s [50 mL/min]. Patients with multiple primary carcinomas or distant metastases at presentation were excluded from this study, as were patients whose tumor represented a second primary carcinoma.

All patients were evaluated in a multidisciplinary clinic by a head and neck surgeon, radiation oncologist, and medical oncologist. Staging was done in accordance with criteria established by the American Joint Committee for Cancer Staging. Patients were followed monthly during treatment, and responses were quantified by clinical and radiographic examinations. Primary-site and lymph-node responses were scored separately, and overall response was determined according to the site showing the lesser response. A complete response was defined as the disappearance of all clinically or radiographically evident tumor. A partial response was defined as a reduction of greater than 50% in the product of the two greatest perpendicular diameters of all measurable disease. No response was defined as any response less than a partial response. After completion of chemotherapy patients had surgery and radiation therapy or radiation therapy alone according to the treatment plan.

The PFL regimen included cisplatin, 25 mg/m^2 body surface area daily administered on days 1 through 5 (5 days total); 5-fluorouracil, 800 mg/m^2 body surface area daily on days 2 through 6 (5 days total); and leucovorin, 500 mg/m^2 body surface area daily days 1 through 6 (6 days total) (Figure 1). All drugs were administered by continuous intravenous infusion and repeated once every 28 days. Cisplatin was mixed in 500 mL of normal saline daily. Hydration before cisplatin infusion was not mandatory. Leucovorin and 5-fluorouracil were mixed together in 3 litres of normal saline daily, and the solution was shielded from light. The duration of 5-fluorouracil and leucovorin infusion was decreased by 1 day in patients who experienced grade 3 mucositis or diarrhea during the preceding cycle. No dose reduction for cisplatin was planned; however, PFL therapy was terminated if serum creatinine increased by more than 25% over the value obtained before treatment or if the creatinine clearance decreased to 0.66 mL/s (40 mL/min) or to less than 70% of the baseline level. The use of steroids as antiemetics was not permitted.

A maximum of three cycles were administered before local-regional therapy. In the first 23 patients, restaging after two cycles led either to the administration of a third cycle of PFL if a partial response was shown or to the termination of PFL if the response was either complete or less than partial (no response). Subsequently, all patients received three cycles, except patients who did not respond after rwo cycles. After completion of chemotherapy, patients were treated with surgery and radiation therapy or radiation therapy alone. If surgical resection was not to follow chemotherapy, a biopsy to assess primary-site pathologic response was attempted. A more rigorous biopsy program, however, began in 1989, and 13 of 16 (81%) patients have had biopsies thus far. In patients who showed a partial response, areas suspected to contain residual tumor were biopsied. In patients who achieved a complete response, the tumor bed, outlined by tattoo before the start of therapy, was biopsied. This study was approved by the Investigational Review Board of the Dana Farber Cancer Institute. Signed informed consent was obtained before treatment.

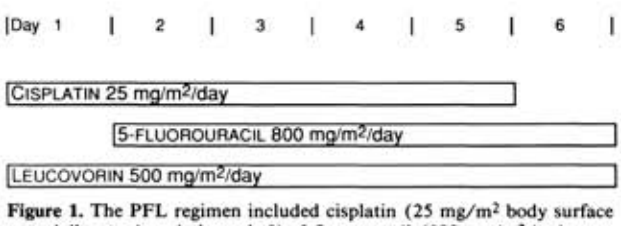

Figure 1. The PFL regimen included cisplatin (25 mg/m^2 body surface area daily, on days 1 through 5); 5-fluorouracil (800 mg/m^2 body surface area daily, on days 2 through 6), and leucovorin (500 mg/m^2 body surface area daily, on days 1 through 6). All drugs were administered by continuous intravenous infusion and repeated once every 28 days. Blood samples for the measurement of serum levels of leucovorin and (6S)5-methyltetrahydrofolate were obtained just before initiation of chemotherapy and 18 hours thereafter.

Assay for Leucovorin and 5-Methyltetrahydrofolate

Two 20-mL blood samples were collected, the first just before treatment was begun and the second after 18 hours of cisplatin and leucovorin infusion. They were spun in a clinical centrifuge to obtain serum, which was kept at 193 deg Kelvin before analysis. For the measurement of serum leucovorin and 5-methyltetrahydrofolate concentrations, a series of standards were prepared containing 0 to 26 μmol/L of leucovorin and 0 to 56 μmol/L of 5-methyltetrahydrofolate in a total volume of 120 μL of serum from an untreated donor. Before their addition, no leucovorin or 5-methyltetrahydrofolate could be detected in the donor serum. A 100-μL aliquot of each standard or patient serum sample was added to 200 μL of methanol. The precipitated proteins were removed by 15 minutes of spinning on a model 235B microcentrifuge (Fisher Scientific Company, Medford, Massachusetts). To 240 μL of each supernatant was added 60 μL of 100 μmol/L 4-(*N,N*-dimethylamino)benzoic acid as an internal standard. The samples were analyzed by high-performance liquid chromatography (32). Relative amounts of the 6R and 6S diastereomers of leucovorin were also measured by chiral high-performance liquid chromatography on two tandem Resolvosil columns (Rainin Instrument Company, Woburn, Massachusetts) (33). Each assay was repeated on four different days.

Results

The characteristics and disease extent of the 35 patients who completed PFL therapy are shown in Table 1. Four patients (11%) had stage III and 31 (89%) stage IV (M0) squamous cell carcinoma of the head and neck: 27 (77%) had T3 or T4 lesions, 25 (71%) had N2 or N3 nodes 3, and 18 (51%) had T3-4N2-3 disease. All 35 patients were evaluable for response and toxicity.

A clinical response to induction therapy with PFL was noted in 28 of 35 (80%) patients, including 23 complete responses (66%; 90% CI, 50% to 79%) and 5 (14%) partial responses. Seven patients (20%) failed to respond to PFL induction chemotherapy. The complete response rate for stage III and IV patients was 4 of 4 and 19 of 31 (61%), respectively. Of the 18 patients with T3-4N2-3 disease, 11 (72%) achieved a complete response. The impact of T- and N-stage on the response to PFL therapy is presented in Table 2. Biopsy specimens of the primary tumor or specimens of definitive resection immediately after PFL therapy were available in 23 patients (19 com-

Table 1. *Characteristics of the Patients Treated with Cisplatin, 5-Fluorouracil, and Leucovorin**

Characteristic	Patients, *n*
Total	35
Sex	
Male	22
Female	13
Performance status†	
0	19
1	16
Primary site	
Oral cavity	8
Oropharynx	15
Hypopharynx	3
Nasopharynx	6
Larynx	2
Paranasal sinuses	1
Primary-site stage	
T1	3
T2	5
T3	13
T4	14
Nodal stage	
Nx	1
N0	7
N1	2
N2a	9
N2b	3
N3a	5
N3b	8
Overall stage	
III	4
IV	31

* Median age, 53 years; range, 26 to 66 years.
† According to the Eastern Cooperative Oncology Group scale.

plete responses, 3 partial responses, and 1 no response). In 12 of 19 (63%) patients who achieved a complete response, no residual tumor was found in the specimen. Of 2 patients classified as having had a partial response based on less than a complete response in the primary site, 1 had no residual tumor in the specimen, whereas a focus of carcinoma in situ was found in the other. Specimens from neck dissection were available in 4 of the 18 patients who achieved a nodal complete response. In 3 of the 4 patients, no evidence of residual disease was found.

Eleven patients received 2 cycles of PFL therapy, and 24 patients received 3 cycles. A complete response was achieved in 9 (26%) patients after 2 cycles and in 14 (40%) additional patients with a third cycle of PFL. The toxicities of induction therapy with PFL are shown in Table 3. A total of 94 cycles of PFL therapy were administered. Dose reduction (5-fluorouracil and leucovorin) for toxicity after the first cycle was necessary in 11 (31%) patients. There was no dose reduction after the second cycle. The commonest toxicity was mucositis, which developed in all but 1 patient. It was painful and debilitating, grade 2 or 3, in 94% of patients during the first cycle. Six patients required in-hospital care for mucositis. Mucositis usually began 1 to 2 days after completion of drug administration and lasted from 3 to 10 days. With subsequent dose reduction, however, mucositis did not develop or was substantially less severe. The incidence of diarrhea was 17%. Diarrhea tended to be mild and to resolve spontaneously, although 1 patient required hospitalization for diarrhea and diffuse abdominal pain. A patchy macular or maculopapular rash was seen in 26% of patients. It was mildly pruritic in some patients, but completely asymptomatic in most. Exfoliative dermatitis, requiring no specific treatment, occurred in 3 patients. Myelosuppression was mild. Fever associated with neutropenia was seen in two patients. Although the platelet counts had decreased to below 50×10^9/L (50 000 cells/μL) in 6 (18%) patients during the first cycle (half of whom had counts below 20×10^9/L (20 000 cells/μL), no bleeding episodes occurred. Conjunctivitis was not seen. Other noted toxicities included prolonged pancytopenia (leukocyte count $< 2 \times 10^9$/L [$<$ 2000 cells/μL] for more than 14 days) in one patient. Cisplatin had to be discontinued in 1 patient on the second day of the final cycle because of a 30% rise in serum creatinine and a corresponding decrease in creatinine clearance. Mild peripheral neuropathy developed in 1 patient. There were no treatment-related deaths.

Table 2. *The Impact of Tumor and Node Stage on Complete Response to Induction Chemotherapy with Cisplatin, 5-Fluorouracil, and Leucovorin*

Stage	Patients			
	Number	With Clinical Complete Response	With Evaluable Specimens	With Pathologically Confirmed Complete Response
		n(%)	*n*	*n*(%)
T0	0	...	NA*	NA
T1	3	3(100)	0	NA
T2	5	4(80)	3	1(33)
T3	13	10(77)	6	4(67)
T4	14	10(71)	10	9(90)
Total	35	27(77)	19	14(74)
Nodal				
Nx	1	...	NA	NA
N0	7	...	NA	NA
N1	2	2(100)	NA	NA
N2	12	10(83)	4	3(75)
N3	13	6(46)	NA	NA
Total	35	18(67)†	4	3(75)

* NA = not available.
† Of 27 patients, 18 had evaluable neck disease ($\geq$ N0).

The mean serum leucovorin level for 7 patients after 18 hours of treatment was 34.3 ± 1.5 μmol/L. The mean molar fraction of the drug in the form of the active 6S isomer was only 0.08 ± 0.005. In contrast, serum concentrations of (6S)5-methyltetrahydrofolate were 9.2 ± 0.6 μmol/L in the same patient samples, and no (6R)5-methyltetrahydrofolate was found. Sera obtained before treatment did not contain leucovorin diastereomer and endogenous 5-methyltetrahydrofolate was not detectable by our assay.

Table 3. *Toxicity Seen with Continuous Infusion Cisplatin, 5-Fluorouracil, and Leucovorin*

Type of Toxicity	Cases, n (%)	
	First Cycle (n = 35 cycles)	All Cycles (n = 94 cycles)
Gastrointestinal (Grade ≥ 2)		
Mucositis	33(94)	34(36)
Diarrhea	6(17)	11(12)
Nausea and vomiting	13(37)	20(21)
Hematologic		
Leukocyte nadir		
≤ 2 × 10^9/L (2000 cells/μL)	8(23)*	13(14)†
≤ 1 × 10^9/L (1000 cells/μL)	3(6)*	5(5)†
≤ 0.5 × 10^9/L (500 cells/μL)	...	...
Platelet nadir		
≤ 50 × 10^9/L (50 000 cells/μL)	6(18)*	11(12)†
≤ 20 × 10^9/L (20 000 cells/μL)	3(9)*	6(6)†
Dermatologic		
Rash	9(26)	16(17)
Renal		
Serum creatinine > 152 μmol/L (2.0 mg/dL)	...	1(1)
Peripheral neuropathy, *n(%)*	...	1(1)

* Data not available for 1 of 35 cycles.
† Data not available for 2 of 94 cycles.

Discussion

We describe the activity and toxicity of a new regimen combining continuous infusion high-dose leucovorin with 5-fluorouracil and cisplatin in the therapy of patients with advanced, previously untreated stage IV squamous cell carcinoma of the head and neck. Of 35 patients treated, a complete clinical response was achieved in 66%. Moreover, of 27 patients with a complete clinical response at the primary site, a complete response was confirmed pathologically in 14 of 19 (74%) patients who had biopsies or resection after PFL therapy. Analysis of the impact of the T-stage on the response to PFL therapy appears to indicate that a high rate of pathologically confirmed complete response can be achieved in T3 and T4 lesions. Although encouraging, this finding should be interpreted with caution because of the small number of patients in each stage subset.

The results achieved with PFL therapy compare favorably with the complete response rate of 23% ($P < 0.001$) seen in a group of 201 historical controls with stage IV squamous cell carcinoma of the head and neck who were treated at the Dana Farber Cancer Institute with previous cisplatin-based induction regimens, including PF. The toxicity of PFL, although common, consisted predominantly of moderate to severe mucositis, which in no case appeared to be life-threatening. Dose reduction for toxicity was necessary after the first cycle in 31% of patients. This controlled or decreased the toxicity seen in subsequent cycles.

Given the heterogeneity of squamous cell carcinoma of the head and neck in terms of anatomic location, histology, and tumor volume, these apparently superior results should be interpreted with caution. Although the response rate is promising, it cannot be determined from this phase II study whether the present drug combination is in fact superior to PF. It also cannot be concluded whether the addition of leucovorin to PF is superior to a PF regimen that would use higher-than-standard doses of 5-fluorouracil or higher doses of cisplatin administered by continuous infusion (125 mg/m^2 total dose instead of the usual 100 mg/m^2).

As in the design of most other induction chemotherapy trials for this disease at the Dana-Farber Cancer Institute, the number of cycles administered in the present study was individualized initially (first 23 patients), with two or three cycles of PFL therapy given depending on the observed response. However, the present experience indicates that three cycles of PFL therapy are important in achieving a high rate of complete response. Vokes and colleagues (34) have reported their experience with a regimen combining bolus cisplatin (100 mg/m^2), 5-day continuous infusion of 5-fluorouracil (800 mg/m^2 daily), and oral leucovorin (100 mg every 4 hours for 5 days). Twenty-two previously untreated patients with stage III and IV squamous cell carcinoma of the head and neck received this drug combination for two cycles. The overall and complete response rates were 91% and 32%, respectively. Whereas the overall response rate is similar to that which we report, the complete response rate observed by these investigators was lower. The complete response rate in our study after two cycles of PFL therapy was 26% and increased to 66% after the administration of an additional third cycle. It appears that at least three cycles of PFL therapy should be given to maximize the complete response rate. An increase in the rate of complete response with three cycles, as compared with two cycles of chemotherapy, has been reported previously (10, 16).

Many mechanisms may contribute to the high complete response rate achieved with PFL therapy. Enhancement of 5-fluorouracil cytotoxicity by leucovorin is probably a major factor (22-26, 35-39). This modulation of 5-fluorouracil is an outcome of the cellular metabolism of both drugs and the subsequent interaction of their metabolites. The 5-fluorouracil is converted enzymatically to 5-fluorodeoxyuridylic acid (FdUMP), whereas leucovorin forms (6R)5,10-methylenetetrahydrofolic acid (CH_2THF). These intracellular metabolites inhibit the target enzyme thymidylate synthase by forming a covalent ternary complex, TS-FdUMP-CH_2THF (22, 38, 39). Under

normal conditions, FdUMP binding to the target enzyme is reversible with a half-life of 2 hours or less (40, 41). However, when a large excess of CH_2THF is present, ternary-complex formation is essentially complete and the dissociation half-life exceeds 35 hours (40, 41). This results in a marked reduction in the amount of thymidylate synthase available to catalyze the conversion of dUMP to dTMP, which is essential for normal DNA synthesis.

The administration of cisplatin and 5-fluorouracil by continuous infusion and the delayed initiation of 5-fluorouracil are other factors that may contribute to the activity of PFL (28-31), although one study found that cytotoxicity is increased when bolus cisplatin is administered after bolus 5-fluorouracil in a murine model (42). The initiation of leucovorin infusion 24 hours before 5-fluorouracil in the present regimen is based on the assumption that a period of time may be necessary to allow sufficient intracellular accumulation of 5, 10-methylenetetrahydrofolic acid, thus potentiating 5-fluorouracil cytotoxicity from the start of its infusion. The start of cisplatin before 5-fluorouracil in the PFL regimen is based on in-vitro data that indicate that cisplatin enhances the cytotoxicity of subsequent 5-fluorouracil administration (43). The combination of cisplatin and 5-fluorouracil was shown in vivo to be highly synergistic (44).

Analysis of patient sera may clarify the mechanisms responsible for the activity of PFL. In our study, serum samples from seven patients were obtained before and 18 hours after chemotherapy. Concentrations of leucovorin and (6S)5-methyltetrahydrofolate before treatment were below detection limits. After 18 hours of cisplatin and leucovorin infusion, however, the mean leucovorin level was 34.3 ± 1.5 μmol/L. This result is consistent with the reported mean plasma steady-state level of 37.5 ± 8.4 μmol/L during continuous intravenous infusion of leucovorin at 500 mg/m^2 daily for 5.5 days observed in two patients with colorectal carcinoma and one patient with hepatocellular carcinoma (45). Serum samples in our study, however, contained 9.2 ± 0.6 μmol/L of (6S)5-methyltetrahydrofolate, which contrasts with the steady-state plasma level of 4.8 ± 1.3 μmol/L reported in that study. Although this difference may be related to the fact that our samples were obtained from patients with head and neck cancer, the higher levels of (6S)5-methyltetrahydrofolate seen in our patients possibly derive from the coadministration of cisplatin. Treatment of cells in culture with cisplatin was shown to expand 5,10-methylenetetrahydrofolic acid pools, which in turn could produce an increase in (6S)5-methyltetrahydrofolate because of the activity of 5, 10-methylenetetrahydrofolate reductase (43).

Analysis of the individual 6R and 6S diastereomers of leucovorin showed that a molar fraction of only approximately 0.08 ± 0.005 of the total serum content of the drug is in the form of the active 6S diastereomer, which is consistent with the previously reported molar fraction of 0.058 ± 0.012 (45). This may be due to the more rapid metabolism of the 6S isomer. The influence of (6R)leucovorin on response and toxicity is unknown and must be clarified in future studies (46).

In conclusion, PFL appears to be a highly active chemotherapy regimen in patients with previously untreated, advanced squamous cell carcinoma of the head and neck. The results observed with PFL therapy require further confirmation, and the impact of this regimen on local tumor control and disease-free and overall survival remains to be determined in a randomized study. We shall continue to study this drug combination to address these questions and further the understanding of its mechanisms of action.

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