Cancer Chemother Pharmacol (1989) 24: 41–44

Cancer Chemotherapy and Pharmacology

Phase II study of Adriamycin with sequential methotrexate and 5-fluorouracil (AMF) in Gastric carcinoma

Jaffer A. Ajani, Pamela Goudeau, Bernard Levin, Jack S. Faintuch, James L. Abbruzzese, Bruce M. Boman, and Mahesh D. Kanojia

Department of Medical Oncology, Division of Medicine, The University of Texas M. D. Anderson Cancer Center at Houston, Houston, TX 77030, USA

Summary. The purpose of this study was to evaluate the response rate, methotrexate plasma levels, and toxicity of a three-drug regimen in patients with gastric carcinoma. A total of 37 patients with advanced measurable adenocarcinoma of the stomach were treated with Adriamycin, methotrexate, and 5-fluorouracil (AMF). Adriamycin and methotrexate were given as i.v. infusions on day 1; 24 h following methotrexate administration, patients received an i.v. infusion of 5-fluorouracil concomitantly with oral leukovorin factor (given over 48 h). Methotrexate levels were monitored regularly in all patients, and courses were repeated every 3 weeks. The median dose levels per course were 50 mg/m^2 (range, 40–60 mg/m^2) for Adriamycin, 1,000 mg/m^2 (range, 650–1,250 mg/m^2) for 5-fluorouracil, and 500 mg/m^2 (range, 160–625 mg/m^2) for methotrexate. Of 36 evaluable patients, 8 (22%) achieved an objective response, including 1 complete remission. Stable disease was noted in 11 patients and a minor tumor regression occurred in 1. The median survival duration of all patients was 6 months (range, 2–31+ months). AMF was well tolerated; toxicities were mild to moderate, most frequently involving nausea and vomiting, mucositis, and neutropenia with or without fever. There was no death directly attributable to chemotherapy. Although the AMF regimen used a well-documented preclinical concept of synergism between methotrexate and 5-fluorouracil, response and survival results suggest a modest activity of this combination in patients with gastric cancer. Better preclinical models are necessary for the development of effective combination chemotherapy.

Introduction

Although the incidence of gastric carcinoma has been declining, it remains eight most common cause of cancer deaths in this country, and the 5-year patient survival has ranged between 8% and 16% for the last three decades [23]. Curative surgery provides the only chance for long-term survival. Neither chemotherapy nor the chemotherapy plus radiation therapy used against advanced disease has often resulted in complete remissions or long-term survivals [11, 12, 15, 16, 19, 21]. Thus, a need exists to develop effective chemotherapeutic combinations.

Offprint requests to: Jaffer A. Ajani

Although the mechanisms of synergy between 5-fluorouracil and methotrexate are not fully understood [1–5, 8], two postulates have been put forward. The first proposes that methotrexate and its polyglutamates inhibit the enzyme dihydrofolate reductase, thus reducing the synthesis of tetrahydrofolate, without which cells cannot synthesize thymidylate from dioxyuridylate and purines. Methotrexate polyglutamates and dihydrofolate polyglutamates can participate in forming stable ternary complexes with fluorodoxyridylate and thymidylate synthetase [4, 8].

The second postulate is that the inhibition of purine synthesis by methotrexate leads to accumulation of the coenzyme phosphoribosylphyrophosphate (PRPP) [5]. PRPP is one pathway by which 5-fluorouracil is converted to its mononucleotides. An increase in the level of PRPP along with an increase in 5-fluorouracil nucleotides results in an increased cell kill [1, 2]. The effect of proper sequencing on the degree of tumor-cell toxicity has been demonstrated in vivo [3] as well as in vitro [18]. Studies comparing the duration of pretreatment exposure times [3, 18] have revealed that the greatest degree of cytotoxicity was achieved with a methotrexate preexposure time of 24 h.

Based on the single-agent activity of Adriamycin and 5-fluorouracil [6, 10, 20] as well as the significant activity of combined Adriamycin and methotrexate [9], we initiated a trial using a combination of Adriamycin, sequential methotrexate, and 5-fluorouracil for patients with measurable, advanced gastric carcinoma. We strove to achieve a therapeutic 24-h methotrexate serum level ($>1\times10^{-6}M$) to assure the highest potential thymidylate synthesis inhibition, purine synthesis inhibition, and cytotoxicity [22].

Patients and methods

Patient selection. Patients with histologic proof of advanced adenocarcinoma of the stomach were entered in this study. Requirements for entry included a performance status of ≤ 2 (Zubrod scale) and a life expectancy of at least 12 weeks. All patients had radiographically measurable disease. No prior chemotherapy for advanced disease was allowed; however, adjuvant chemotherapy that ended 6 months prior to registration was permissible. Moreover, before therapy began, serum creatinine and bilirubin levels were required to be normal, the absolute granulocyte count had to be $\geq 1{,}500$ cells/mm^3, and the platelet count had to be $\geq 100{,}000$/mm^3. All patients gave their written informed consent in accordance with institutional regulations.

Treatment. All patients were hospitalized for the first course of chemotherapy. However, depending upon their understanding, ability, and available social support, some patients received subsequent chemotherapy courses as outpatients. Treatment began with i.v. hydration using 5% dextrose in water with 80 mEq $NaHCO_3$ (1–2 l) until the patient's urine became alkaline (pH ≥ 7.0). The alkalinization process was continued during therapy until the serum methotrexate level was $< 1 \times 10^{-6} M$. Patients receiving outpatient chemotherapy maintained urinary alkalinity by ingesting sodium bicarbonate tablets (1,800–2,000 mg) orally every 6 h. Methotrexate was infused i.v. over 2 h, followed by Adriamycin given as a short i.v. infusion. To reduce cardiac toxicity, Adriamycin was given as a 24-h continuous infusion via a portable pump once patients had achieved a complete or partial response.

At 24 h after the methotrexate infusion, patients received 5-fluorouracil over 1 h and 15 mg leukovorin factor orally every 6 h for a total of eight doses. The starting dose of methotrexate in the first eight patients was 200 mg/m^2; it was subsequently raised to 500 mg/m^2 to obtain consistent 24-h methotrexate serum levels at a micromolar concentration. The starting dose of Adriamycin was 50 mg/m^2 and the 5-fluorouracil dose was 800 mg/m^2. Doses were modified by predetermined criteria based on the toxicity experienced during the preceding course.

Toxic effects were evaluated after each course and response, after every two courses. Weekly complete blood counts, differential counts, and platelet counts were obtained. Liver and renal function tests were repeated prior to each course. Serum methotrexate levels were determined at 24-h intervals until the level dropped to $< 1 \times 10^{-6} M$. The criteria for defining objective response were standard, except that palpable hepatomegaly was not considered to be a measure of response.

Results

Of 38 patients, 1 did not receive chemotherapy after registering; thus, the following analysis reflects the remaining 37 patients, whose characteristics are listed in Table 1. The first eight patients, who received an initial methotrexate dose of 200 mg/m^2, are designated as group A, and the 29 subsequent patients, who received an initial methotrexate dose of 500 mg/m^2, are designated as group B. One patient had received prior 5-fluorouracil for advanced disease (the only protocol violation); two others had received adjuvant chemotherapy.

The treatment characteristics are summarized in Table 2. The median dose levels per course were 50 mg/m^2 (range, 40–60 mg/m^2) for Adriamycin, 1,000 mg/m^2 (range, 650–1250 mg/m^2) for 5-fluorouracil, and 500 mg/m^2 (range, 165–625 mg/m^2) for methotrexate [a median of 200 mg/m^2 (range, 165–300 mg/m^2) for group A and a median of 500 mg/m^2 (range, 400–625 mg/m^2) for group B]. Four patients received a cumulative Adriamycin dose of ≥ 450 mg/m^2 (450, 550, 710, and 790 mg/m^2).

Therapeutic response

Of 37 patients, 36 were evaluable for response. One patient who did not return for evaluation after the second course of chemotherapy was not evaluated for response. One patient achieved a complete response (duration, 16 months) and seven had partial responses (median duration, 8 months; range, 3–14 months); thus, the total response rate was 22% (8/36). One patient had a minor response, and disease remained stable in 11. The median survival of all patients was 6 months (range, 2–31+ months).

The methotrexate levels measured in these patients are listed in Table 3. When the starting methotrexate dose was increased to 500 mg/m^2 (group B), at 24 h the serum levels increased by 48% and at 48 h by 80%, over those in group A patients. In group A, 8 of 27 courses (30%) resulted in 24-h serum methotrexate levels $\leq 1 \times 10^{-6} M$, whereas in group B, only 7 of 75 courses (9%) achieved such levels.

Table 1. Patient characteristics

Caracteristics	All patients	Group A[a]	Group B[b]
Patients (*n*)	37	8	29
Median age, years (range)	51 (25–68)	54 (40–66)	48 (25–68)
Male: female	26:11	5:3	21:8
Performance status			
0	6	1	5
1	25	5	20
2	5	2	3
3	1	0	1
Prior irradiation	2	1	1
Prior chemotherapy	3	2	1
Measurable sites:			
Metastases	32	5	27
Primary	5	3	2

[a] Initial methotrexate dose of 200 mg/m^2
[b] Initial methotrexate dose of 500 mg/m^2

Table 2. Treatment characteristics

Total courses (*n*)	160
Median courses/patient (*n*) (range)	5 (1–16)
Total Adriamycin	
Dose, median (mg/m^2) (range)	250 (40–790)
Percentage of courses with:	
Dose escalation	32
Dose reduction	21

Table 3. Methotrexate levels

Time measured	All patients	Median level ($\times 10^{-6}$ M/ml)	
		Group A[a]	Group B[b]
At 24 h	3.2	2.5	3.7
(range)	(0.9–35.0)	(0.8–15.2)	(0.9–35.0)
Assays (*n*)	102	27	75
At 48 h	0.9	0.5	0.9
(range)	(0.1–7.8)	(0.1–7.8)	(0.1–6.0)
Assays (*n*)	101	26	71
At 72 h	0.6	1.0	0.6
(range)	(0.1–2.6)	(0.1–2.6)	(0.1–2.0)
Assays (*n*)	18	3	15

[a] Initial methotrexate dose of 200 mg/m^2
[b] Initial methotrexate dose of 500 mg/m^2

Table 4. Nonhematologic toxicity of AMF

Type	Patients (*n*)	Courses (*n*)	Toxicity grade[a]		
			1	2	3
Nausea and vomiting	25	82	42	38	2
Mucositis	12	23	13	7	3
Fatigue	11	21	13	4	4
Anorexia[b]	10	17	13	3	1
Infection[c]	6	8	0	5	3
Diarrhea	4	5	3	2	0
Dermatitis	3	3	2	1	0
Confusion[b]	2	2	2	0	0
Fever[c]	2	3	0	2	1
Cardiac toxicity	1	[d]	0	0	1

[a] According to WHO toxicity criteria (see [17])
[b] No provision for grading in WHO criteria
[c] Associated with neutropenia (granulocyte count, $< 1{,}000/mm^3$)
[d] Cumulative toxicity

Toxicity

This chemotherapy regimen was well tolerated by all patients. A total of 160 courses given to 37 patients was evaluated for various toxicities according to the criteria of the World Health Organization [17]. The nonhematologic toxicities are listed in Table 4. No chemotherapy-related death occurred, and we did not observe any grade 4 nonhematologic toxicity. Eight episodes of documented infections with neutropenia (absolute granulocyte count of $< 1{,}000$ cells/mm^3) occurred in six patients. Fever during neutropenia with no documented infection occurred after three courses in two patients. One patient who received a cumulative Adriamycin dose of 790 mg/m^2 developed congestive heart failure that responded completely to conventional therapy.

The lowest median counts were: white blood cells, 2,700/mm^3 (range, 400–11,200 cells/mm^3); granulocytes, 1,200 cells/mm^3 (range, 100–8,000 cells/mm^3); and platelets, 222,000/mm^3 (range, 45,000–696,000/mm^3).

Discussion

Chemotherapy for advanced gastric carcinoma is suboptimal. Combination chemotherapy containing 5-fluorouracil, Adriamycin, and mitomycin-C (FAM) is most commonly prescribed in this country for metastatic disease [16]. Although FAM appears to have a higher response rate than 5-fluorouracil alone, complete remissions with the former are rare and a survival advantage is lacking [7].

Based on the reported evidence of preclinical synergistic cytotoxicity with methotrexate and 5-fluorouracil, a notion also used in the present study, Klein et al. combined methotrexate and 5-fluorouracil with Adriamycin (FAMTX) and reported a high response rate in patients with advanced gastric carcinoma [13, 14]. Among 100 patients treated, 59 achieved an objective response, 12 of them complete [14], and the median survival of all patients was 9 months, longer than that reported in most studies. Encouraged by the results of Klein et al. using FAMTX, the European organization EORTC treated 71 patients using the same dose and schedule. A response rate of 33%, including a 13.4% complete response rate, was reported in 67 evaluable patients [24]; this response rate was lower than that reported by Klein's group. The median survival of all patients in the EORTC study was 6 months.

Our study used entirely different schedule and dose levels of the three drugs tested in the European studies. Our data suggest that despite the 24-h methotrexate pretreatment, AMF had modest activity against gastric carcinoma and resulted in modest survival in these patients. More reliable preclinical models are necessary for better prediction of the clinical activity of chemotherapeutic combinations.

References

1. Benz C, Cadman E (1981) Modulation of 5-fluorouracil metabolism and cytotoxicity by antimetabolite pretreatment in human colorectal adenocarcinoma HCT-8. Cancer Res 41: 994–999
2. Benz C, Schoenberg M, Choti M, Cadman E (1980) Schedule dependent cytotoxicity of methotrexate and 5-fluorouracil in human colon and breast tumor cell lines. J Clin Invest 66: 1162–1165
3. Bertino JR, Sawicki CA, Lindquist CA, Gupta VS (1977) Schedule-dependent antitumor effects of methotrexate and 5-fluorouracil. Cancer Res 37: 327–328
4. Bertino JR, Mini E, Fernandes DJ (1983) Sequential methotrexate and 5-fluorouracil: mechanisms of synergy. Semin Oncol 10: 2–5
5. Cadman ED, Heimer R, Davis L (1979) Enhanced 5-fluorouracil nucleotide formation after methotrexate administration: explanation for drug synergism. Science 205: 1135–1137
6. Comis RL, Carter SK (1974) Integration of chemotherapy into combined modality treatment of solid tumors: III. Gastric cancer. Cancer Treat Rev 1: 221–238
7. Cullinan SA, Moertel CG, Fleming TR, Rubin JR, Krook JE, Everson LK, Windschitl HE, Twito DI, Marschke RF, Foley JF, Pfeifle DM, Barlow JF (1985) A comparison of three chemotherapeutic regimens in the treatment of advanced pancreatic and gastric carcinoma. J Am Med Assoc 253: 2061–2067
8. Fernandes DJ, Bertino JR (1980) 5-Fluorouracil – methotrexate synergy: enhancement of 5-fluorodoxyuridylate binding to thymidylate synthase by dihydropteroyl-polyglutamates. Proc Natl Acad Sci USA 77: 5663-5667
9. Franks CR (1980) Adriamycin and methotrexate in metastatic gastric cancer: a pilot study. Clin Oncol 6: 309–315
10. Gastrointestinal Tumor Study Group (1979) Phase II–III chemotherapy study in advanced gastric cancer. Cancer Treat Rep 63: 1871–1876
11. Gastrointestinal Tumor Study Group (1982) A comparison of combination chemotherapy and combined modality therapy for locally advanced gastric carcinoma. Cancer 49: 1171–1177
12. Holbrook MA (1974) Gastric cancer treatment principles: radiation therapy. J Am Med Assoc 228: 1289–1290
13. Klein HO, Wickramanayake PD, Dieterle F, Mohr R, Oerkermann H, Gross R (1983) High-dose MTX/5-FU and adriamycin for gastric cancer. Semin Oncol 10: 29–31
14. Klein HO, Wickramanayake PD, Farrokh GR (1986) 5-Fluorouracil (5-FU), adriamycin (ADM), and methotrexate (MTX)-a combination protocol (FAMTX) for treatment of metastasized stomach cancer. Abstract Proc Am Soc Clin Oncol 5: 8
15. Levi JA, Dalley DN, Aroney RS (1979) Improved combination chemotherapy in advanced gastric cancer. Br Med J [Clin Res] 2: 1471–1473
16. MacDonald JS, Woolley PV, Smythe T, Ueno W, Hoth D, Schein P (1980) 5-Fluorouracil, doxorubicin, and mitomycin (FAM) combination chemotherapy for advanced gastric cancer. Ann Intern Med 93: 533–536

17. Miller AB, Hoogstraten B, Staquet M, Winkler A (1981) Reporting results of cancer treatment. Cancer 47: 207–214
18. Mini E, Bertino JR (1982) Time and dose relationships for methotrexate, fluorouracil, and combinations of these drugs for maximum cell kill in human CCRF-CEM cells. Abstract Proc Am Assoc Cancer Res 23: 181
19. Moertel CG, Lavin PT (1976) Phase II–III studies in advanced gastric cancer. Cancer Treat Rep 60: 733–737
20. Moertel CG, Lavin PT (1979) Phase II–III chemotherapy studies in advanced gastric cancer. Cancer Treat Rep 63: 1863–1869
21. Moertel CG, Rubin J, O'Connell MJ, Schutt AJ, Wieand HS (1986) A phase II study of combined 5-fluorouracil, doxorubicin, and cisplatin in the treatment of advanced upper gastrointestinal adenocarcinomas. J Clin Oncol 4: 1053–1057
22. Pinedo HM, Zaharko DS, Bull JM, Chabner BA (1977) The relative contribution of drug concentration and duration of exposure to mouse bone marrow toxicity during continuous methotrexate infusion. Cancer Res 37: 445–450
23. Silverberg E, Lubera J (1988) Cancer statistics. CA 38: 5–22
24. Wils J, Bleiberg H, Dalesio O (1986) An EORTC Gastrointestinal Group evaluation of the combination of sequential methotrexate and 5-fluorouracil combined with adriamycin in advanced measurable gastric cancer. J Clin Oncol 4: 1799–1803

Received August 8, 1988/Accepted November 2, 1988