RESEARCH COMMUNICATION

Prognostic Value of Excision Repair Cross-complementing Gene 1 Expression for Cisplatin-Based Chemotherapy in Advanced Gastric Cancer

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Abstract

Background: Association of excision repair cross-complementing gene 1 (ERCC1) expression and treatment response and survival was evaluated in advanced stages of gastric cancer patients who were given different platinum-based chemotherapy. Patients and Methods: Forty-one patients with advanced gastric cancer were enrolled into the study from January 2000 to December 2009. ERCC1 expression was evaluated by immunohistochemistry (IHC). Results: Thirteen of the 41 patients (31%) were shown to have ERCC1 positive lesions. Although the clinical benefit from platin based chemotherapy was the same for ERCC1 positive and negative patients, survival times were statistically significantly better in ERCC1 negative gastric cancer patients. Conclusion: We suggest that IHC studies for ERCC1 may be useful in prediction of the clinical outcome of advanced gastric cancer patients treated with platin-based chemotherapy.

Key Words: Advanced gastric cancer - cisplatin based chemotherapy - ERCC1

Asian Pacific J Cancer Prev, **11**, 181-185

Introduction

The majority of gastric cancer patients in Turkey have stage III or IV disease at presentation and are therefore candidates for some form of chemotherapy. Currently, 1-year survival rates are <50% in stage IIIA and B disease, and <25% in stage IV disease (Hundahl et al., 2000). On the evidence of four randomized trials of chemotherapy versus best supportive care, chemotherapy confers benefits both in quality of life and in survival. These trials showed survival of 7–12 months with chemotherapy and only 3–4 months without it. Although gastric cancer has been considered as a chemosensitive tumor for many years, no significant progress in its management has resulted within the last two decades. Most responses to chemotherapy are partial and of short duration. Median survival is 7–9 months and survival at 2 years is exceptionally >10% (Glimelius et al., 1997; Catalano et al., 2009) A combination of platinum-based chemotherapy in the treatment of advanced gastric cancer is considered a standard. Identification of patients will benefit from platinum-based therapy, treatment response and survival may increase. New combinations (for example; taxans and irinotecan) with platinum or addition of targeted therapy agents further improve the treatment results in these patients (Wagner et al., 2006).

The excision repair cross-complementing gene 1 (ERCC1) is an excision nuclease within the nucleotide excision repair pathway. ERCC1 forms a heterodimer with XPF. As a unit, they execute the 5' incision into the DNA strand, relative to the site of DNA damage, in the nucleotide excision repair excision process. Studies by Sancar and Reardon show that the 5' excision is the last of several steps that are specific to excision of a platinum-DNA lesion. ERCC1 is one of the 16 proteins that compose the nucleotide excision repair repairosome (Lee et al., 1993; Sancar et al., 2004). Malignancies in which mRNA expression of ERCC1 is directly related to clinical outcome in response to DNA damaging chemotherapy include lung cancer, head and neck cancers, gastric cancer, colorectal cancer, and ovarian cancer (Codegoni et al.,1997; Metzger et al.,1998; Britten et al., 2000; Shirota et al.,2001; Lord et al., 2002; Olaussen et al., 2006).

In the present study, assosiation of ERCC1 expression and treatment response and survival was evaluated in advanced stages of gastric cancer patients who were given different platinum-based chemotherapy.

Patients and Methods

Eligibility criteria

All patients in this study had histologically confirmed adenocarcinoma of the stomach. They had unresectable metastases with bidimensionally measurable lesion. These

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patients were treated by cisplatin-based chemotherapy. Patients were selected for this analysis primarily on the basis of availability of adequate tissue for study. Clinical data were obtained by retrospective chart review. All patients had a performance status less than or equal to two according to the Eastern Cooperative Oncology Group scale, adequate bone marrow and renal function, and age between 18 and 79 years.

Exclusion criteria included the presence of central nervous system metastases, serious or uncontrolled concurrent medical illness, and a history of other malignancies. The study was reviewed and approved by the local ethics committee.

Treatment protocols

In twenty patients (49%), ECU (On day 1, Cisplatin (60 mg/m^2) was administered by intravenous (i.v.) infusion in 1000 ml of normal saline over 2 h and epirubicin (50 mg/m^2) was administered by i.v. short infusion and urasil tegafur (UFT) was administered by oral 1-14 days every three weeks. In seven patients (19%), CI (On d 1, 100 mg/m^2 CPT-11 was administered by intravenous infusion for 90 min, followed by a 2 h infusion of 70 mg/m2 cisplatin, with adequate hydration, with a 2 h interval between CPT-11 and cisplatin administration and this treatment was repeated every 3 wk for six courses). In thirteen patient (32%), modified DCF (On day 1, Docetaxel (60 mg/m^2) was administered by i.v. infusion over 1 hour, followed by Cisplatin (60 mg/m^2) was administered by intravenous (i.v.) infusion in 1000 ml of normal saline over 2 hours, Folinic acid (400mg/m^2) was administered by i.v. infusion over 2 hours and 5-Fluorourasil (2400 mg/m^2) was administered by i.v. infusion over 48 hours. This treatment was repeated every 3 wk for six courses).

Immunohistochemical staining for ERCC1

We used a standard protocol for the immunostaining of the samples. In brief, for epitope re- trieval, specimens were exposed to 10 mM cit rate buffer (pH 6,0) and heated for 30 minutes in a water bath. Tumor sections were incubated for 60 minutes with a monoclonal antibody specific against the full-length human ERCC1 protein at a 1:100 dilution (mouse, clone 8F1, Neomark- ers). Antibody binding was detected by means of an ABC kit with NovaRED as the substrate (Vec-tastain Elite, Vector Laboratories) and Mayers hematoxylin as the counterstain. Sections of normal tonsil tissues were included as external positive controls, and stromal cells surrounding the tumor area served as internal positive controls. An investigator who was unaware of clinical data independently evaluated ERCC1 staining under a light microscope at a magnification of 400x. They recorded whether tumor or stromal cells expressed ERCC1. The expression of ERCC1 gene, as determined by the IHC staining, was dividen into two groups according to grading the proportion of nuclei that were stained in the tumor cells. The grading system was as follows: If immunoreactivity was noted in less 10% of the tumor cells, then we defined this as negative; If the immunoreactivity was 10% or more of the tumor cells, then we defined this as positive (Shirota et al., 2001; Olaussen et al.,2006).

Follow-up, evaluation and assessment of response

Patients who received at least three cycle of treatment were considered evaluable for response. The eligibility and suitability for assessment of the subjects and the response to treatment were reviewed by a radiologist externally. Patients who achieved a complete response (CR) or a partial response (PR) and patients with stable disease (SD) were accepted as having clinical benefit (CB) from treatment. Objective responses (OR) were determined according to WHO criteria. The progression free survival (PFS) was estimated from the date of the first cycle to the first evidence of disease progression. Overall Survival (OS) was estimated from the date of the first cycle to the date of death or last follow-up.

Stastistical analysis

The duration of survival and the median and mean event times (95% confidence interval [CI]) were estimated according to the Kaplan–Meier method with SPSS statistical 15.0 software package program. The differences in time distributions between groups were tested for statistical significance using the log-rank test. $P < 0.05$ was considered statistically significant.

Results

Forty-one patients were enrolled into the study from January 2000 to December 2009. All patients characteristics who were given chemotherapy and had avaliable records are shown on Table 1. Thirteen of 41 patients (31%) were shown to have ERCC1 positivity with IHC. Treatment responses were shown on table 2 according to staining ERCC1. No statistically significant difference observed in terms of treatment response between two gruops (P=0.71).

The median progression free survival (PFS) for all ERCC1 positive and negative patients were 5±0,9 mo

Table 1. Patient and Treatment Characteristics

Characteristic		No (%) of patients	
Total 41	Male	30	(73)
	Female	11	(27)
Age (Median)	52 yr (32–74)		
Type of chemotherapy (%)	ECU	20	(48)
	CI	8	(20)
	mDCF	13	(32)
Histopathology	Diffuse type	15	(37)
	Intestinal type	26	(63)
Initial Stage	III	4	(10)
	IV	37	(90)
Region of metastasis	Liver	27	(66)
	Lung	6	(15)
	Ovary	3	(8)
	Peritoneal	9	(23)
	More than one	12	(30)
Performance status (ECOG)	1	34	(83)
	2	7	(17)

Total no of chemotherapy (Median) (Cycles) 6 (2 - 9) ; E C U , Epirubicin, Cisplatin, UFT; CI, Cisplatin and Irinotecan; mDCF, Docetaxel, Cisplatin, 5-FU

Table 2. Response to Treatment according to ERCC1 Positivity and Chemotherapy Combination

ERCC1 Staining	ECU n (%)		CI n (%)		mDCF n (%)		Total n (%)	
	+	-	+	-	+	-	+	-
Total	6 (30)	14 (70)	3 (38)	5 (62)	4 (31)	9 (69)	13 (31)	28 (69)
Partial response	1 (17)	4 (29)	1 (33)	1 (25)	2 (50)	3 (33)	4 (31)	8 (29)
Stable disease	3 (50)	5 (36)	1 (33)	2 (50)	1 (25)	4 (44)	5 (38)	11 (39)
Progression	2 (33)	5 (36)	1 (33)	2 (50)	1 (25)	2 (22)	4 (31)	9 (32)
Overall response	1 (17)	4 (29)	1 (33)	1 (25)	2 (50)	3 (33)	4 (31)	8 (29)
Clinical benefit	4 (67)	13 (65)	2 (67)	3 (75)	3 (75)	7 (77)	9 (69)	19 (68)

(%95 CI 3,2–6,8) and 8±1,2 mo (%95 CI 5,7–10,3), respectively (P=0,17). Six and 12 mo progression free survival rates for ERCC1 positive and negative patients were 38%, 0% and 55%, 20%, respectively. The median overall survival (OS) for ERCC1 positive and negative patients were 8±0,9 mo (%95 CI 6,2–9,8) and 9±1,6 months (%95 GA 5,8–12,2), respectively (P=0,035). Six and 12 months overall survival rates for ERCC1 positive and negative patients were 84%, 18% and 92%, 35% respectively (Figure 1 a and b).

In patients who were given ECU combination chemotherapy, the median survival for ERCC1 positive and negative patients were 6±0,5 mo (%95 CI 5,1–6,9) ve 8±0,3 mo (%95 CI 7,4–8,6), respectively (p=0,064). In patients who were given CI combination chemotherapy, the median survival for ERCC1 positive and negative patients were 8±3,3 mo (%95 CI 1,6–14,4) and 13±1,1 mo (%95 CI 10,9–15,1), respectively (P=0,014). In patients who were given mDCF combination chemotherapy, the median survival for ERCC1 positive patients was 8 mo and not reached for ERCC1 negative patients (P=0.53).

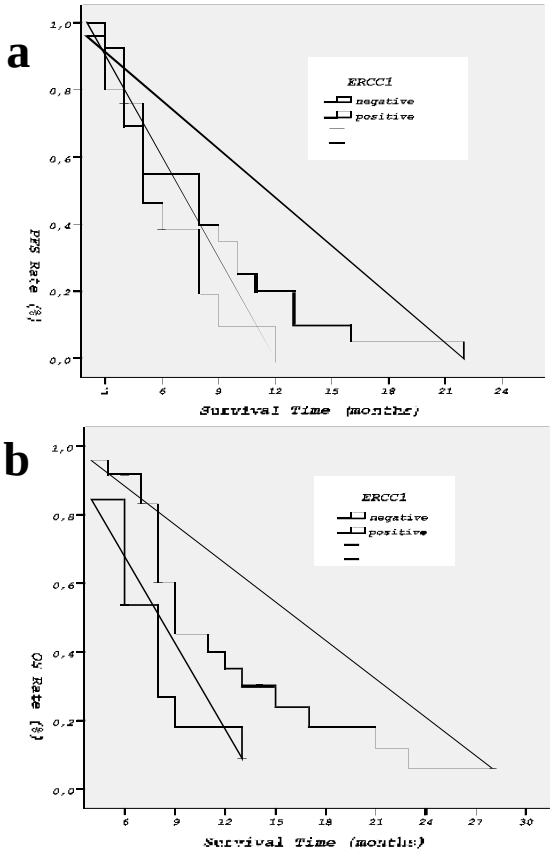

Figure 1. Kaplan-Meier Survival Curves according to ERCC1 Positivity. a) Progression free; b) Overall

Discussion

There is no clear standard of treatment in gastric cancer. Many single-agent or combination chemotherapy regimens have been used. The drug 5-FU remains one of the most popular chemotherapy agents for gastric cancer, and has been the cornerstone of combination regimens such as FAMTX, ELF (etoposide, leucovorin and 5-FU) and ECF (epirubicin, cisplatin and continuous infusion 5-FU). Typically, response rates (RRs) with chemotherapy are in the region of 20% to 30%. In the previous studies, The highest recorded RR is the 45% seen with platin based chemotherpy (ECF) in the randomized trial versus FAMTX, which had a significantly lower 21% RR (Waters et al.,1999).

The limited role of cisplatin for gastric cancer has been thought to be due to cancer's mecanism of resistance, i.e. ERCC1 gene. The ERCC1 gene prevents mutation and other injuries to the DNA via the nucleotide excisionand repair pathway, and the pathway is essential for the repair of cisplatin- DNA adducts (Matsubara et al., 2008).

In our study, clinical benefit from platin-based chemotherapy was similar in ERCC1 negative and positive patients (68% vs. 69%, respectively). But OS time was statistically significantly better in ERCC1 negative gastric cancer patients although the PFS time was not (OS; 8 mo and 9 mo, PFS; 5 mo and 8mo, respectively). This may show that chemotherapy response was more durable in ERCC1 negative patients. Thirteen of 41 patients (31%) were shown to have ERCC1 positivity with IHC. This rate is similar to reported in other gastric cancer studies which evaluted ERCC1 positivity. This means that sixty-nine percent of gastric cancer patients may more benefit from platin-based chemotherapy (Matsubara et al., 2008). The addition of docetaxel or irinotecan to a doublet including cisplatin, especially in patients with ERCC1 negative gastric cancer may provide better results. In our previous phase II study, nine months OS was obtained with cisplatin and irinotecan combination (Altinbas et al., 2005). In this study, the highest survival have been obtained in the patients who were given irinotecan and cisplatin combination and ERCC1 negative (13 mo). According to this study, ERCC1 negative patients benefit more from this combination.

UFT has similar efficacy to continuous infusion 5-FU and improved tolerability but does not have the inconvenience and associated side-effects of catheterization and portable infusion devices. UFT therefore represents a logical replacement for 5-FU in chemotherapy regimens for the treatment of advanced gastric cancer (Aykan et al., 2008). Table 3 summarizes

Table 3. Clinical Studies Conducted on Patients with Gastric Cancer treated with UFT, Epirubicin and Cisplatin

Author	Chemotherapy regimens		n	OS (mo)	Response (%)
Jeen et al.	E (50mg/m^2 day 1), C (60mg /m^2 day 1), UFT and LV (360 and 45 mg /m^2/days 1–21 days) every 28 days.		47	15	(43)
Idelevich et al.	E (50mg/m^2 day 1), C (60mg /m^2 day 1), UFT and LV (300 ve 30 mg /m^2/day 1–21 days) every 28 days.		39	10	(38)
Chaves et al.	E (50mg/m^2 day 1), C (60mg /m^2 day 1), UFT 300 mg /m^2/day 1–21 days) every 28 days.		16	10	(19)
Our study	E (50mg/m^2 day 1), C (60mg/m^2 day 1) and UFT (300mg /m^2/day 1–14 days) every 21 days.	ERCC1 positive	6	6	(17)
		ERCC1 negative	14	8	(29)
		Total	20	8	(25)

C, cisplatin; E, epirubicin

the studies of combinations with cisplatin, epirubicin and UFT (Jeen et al.,2001; Malet-Martino et al., 2002; Idelevich et al., 2007). In our study nearly half of the all patients received this combination regimen and the results were similar to others in terms of efficacy and survival. While the results were better in three studies using the similar combination with leucovorin, in a small study conducted without leucovorin the results were similar to ours. In all of the four studies the regiment was well tolerated. In our study the results tend to be better for response and survival in patients with negative ERCC1 staining, although the difference was not stastically significant. (Treatment response; 29% vs. 17% and OS; 8 vs. 6 mo, respectively) Given these facts ECU combination was a well tolerated regiment and had similar efficacy with other combinations. Addition of leucovorin to this combination may improve the efficacy. Especially ERCC1 negative patients may more benefit from this combination. In V–325 study, addition of docetaxel to cisplatin and 5-FU combination resulted with improvement in treatment response and survival (25% vs. 37% and 8,6 mo vs. 9,2 mo, respectively). Twelve mo survival was 46%. Grade 3–4 toxicity was noted in 69% of patients in DCF arm (Van Cutsem et al., 2006). In our study, we used a modified form of DCF combination and obtained a response rate of 38%. The treatment was well tolerated and there was no early discontinuation related with toxicity (Grade 3-4 toxicity 50%). Twelve mo survival was 46% and median overall survival was not reached. Twelwe mo survival for ERCC positive and negative patients was 33% and 58%, respectively. Although response to treatment was similar in both groups, ERCC1 negative patients benefited more.

With all of the three combination chemotherapies there was no difference in treatment response in favour of ERCC1 negative patients, however survival results tended to be better. Large randomized trials are warranted to recommend non-platin containing regiments to ERCC1 positive patients with advanced gastric cancer. ERCC1 expression was evaluated by IHC and meaningful results were found in these studies. IHC may be preferred as it is cheap and easy method (can be applied almost every pathology laboratory) for this evaluation.

In conclusion, although this study was small in sample size, we were able to find a correlation between clinical outcome and the expression of ERCC1. We suggest that immunohistochemical studies for ERCC1 may be useful in prediction of the clinical outcome of advanced gastric cancer patients treated with platin-based chemotherapy.

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