Sphincter Preservation and Pathologic Response in Rectal Cancer Undergoing Neoadjuvant Chemoradiation with and without Oxaliplatin According to Thymidilate Synthase (TS) Expression

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ABSTRACT

Background

Oxaliplatin (OX) significantly enhanced the antitumor activity of 5-FU in patients with advanced colorectal cancer and recently some phase II trials have evaluated the feasibility and efficacy of oxaliplatin in neoadjuvant setting for treatment of locally advanced rectal cancer. On the other hand various studies have demonstrated that the overexpression of thymidylate synthase (TS) can induce resistance to 5-FU in colorectal carcinoma. The aim of this study was to assess the value of TS expression as a predictive factor in the efficacy of neoadjuvant chemoradiation with and without oxaliplatin in rectal cancer.

Materials and Methods

This study was performed in 61 patients (that ultimately 50 patients had including criteria) with locally advanced rectal adenocarcinoma that inferior margin of the tumor had to be located no farther than 6 cm from the anal verge. Preoperative radiotherapy was delivered to the pelvis with CO 60 to 50/4 Gy. All patients received simultaneous chemotherapy: 5-fluorouracil (5-FU), 300 mg/square meter i.v. 24 h infusion during radiotherapy on days 1-5 every week. Thirty patients received oxaliplatin 50-60 mg /square meter weekly during radiotherapy.TS expression was assessed by immunohistochemical staining technique in pretreatment specimen, and the patients were categorized into TS (+) and TS (-) groups.

Results

A total of 23 of 50 tumors showed TS positive status at biopsy (46 %). Overall 36 patients (72%) achieved pathologic response (40% complete and 32% partial) that was significantly better in the TS (-) group than in the TS (+) group (85.1 vs 56.5%, p=0.024) and in the OX (+) group than in the OX (-) group (86.6 vs 50%, p=0.005). Among TS (-) patients there was no difference in pathologic response (88.2 vs 80%, p=0.561) or sphincter preservation (76.4 vs 80%, p= 0.831) as a result of whether oxaliplatin therapy was carried out or not. But among the TS (+) patients there was a significant gain in pathologic response (84.6 vs 20 %, p=0.002) and sphincter preservation (84.6 vs 40 %, p= 0.026) in favor of oxaliplatin group.

Conclusions

Our study indicate that oxaliplatin can improves poor outcome of TS positive rectal cancer and TS expression may be used for selecting patients for oxaliplatin containing neoadjuvant chemoradiation protocols that can have major role in the tumor down staging and preservation of sphincter and ultimately better quality of life.

Keywords: Rectal cancer, Oxaliplatin, TS, Pathologic response

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INTRODUCTION

Colorectal cancer is the third most common cause of cancer and the second leading cause of

cancer death.(1), Rectal cancer accounting for 35% of all colorectal carcinomas. But Iran compared to world population is a area with low incidence of CRC (49). Incidence of CRC in Iran (Isfahan province) is increasing and rectum is the most site (61.6%) for CRC (50). In locally-advanced stage rectal cancer treated with surgery alone local recurrence occurs in 20-60% of patients. In this group of patients adjuvant or neoadjuvant radiotherapy with 5-fluorouracil (FUra)-based chemotherapy have improved survival (2) and resulted in a dramatic decline in the rate of local failure from >30% to <10% over the last 20 years.(3), When comparing the benefits of neoadjuvant treatment versus adjuvant, neoadjuvant approaches reduces acute toxicity and decreases tumor bulk, enhances sphincter preservation (7) particularly in locally-advanced low-lying rectal cancer. In this type of rectal cancer if the surgery is performed before chemotherapy resection of sphincter and need to colostomy will be necessary that have major role in the quality of life. But 20-30% of these patients continue to develop distant metastases and despite the use of this protocol (4-6) probably due to ineffective control of micrometastatic disease.

Preclinical studies have showed that oxaliplatin (OXA) is a potent radiosensitising agent (8) and several clinical trials have demonstrated the higher activity of different OXA/FUra/leucoverin (LV) combination as compared to the same regimen of FUra/LV alone in metastatic colorectal cancer (9,10) and even in adjuvant setting.(11), More recently, some phase II trials have demonstrated the feasibility and efficacy of combining oxaliplatin with FUra and RT in neoadjuvant setting for treatment of locally advanced rectal cancer.(12-15)

Thymidylate synthase (TS) is a rate-limiting enzyme in the DNA synthetic pathway and represents the cellular target of the antimetabolite drug 5-fluorouracil (FUra).(16), TS catalyzes the de novo synthesis of 2'-deoxi-thymidine-5'-monophosphate (dTMP) from 2'-deoxi-uridine-5'-monophosphate (dUMP) to supply thymidine for DNA synthesis.(17-19), The inhibition of TS is one of the main mechanisms of the action of fluoropyrimidines, particularly 5-fluorouracil (5-FU). The active metabolyte of 5-FU, 5-fluoro-deoxi-uridine-monophosphate (FdUMP), forms a ternary complex with TS and with a folate co-factor (5,10-methylene-tetrahydro-folate-CH2-THF). This ternary complex is stable and inhibits the activity of the enzyme.(17-19)

Various studies have demonstrated, both in experimental and clinical trials, that the level of expression of this enzyme and the ability to achieve its inhibition are the major determinants of sensitivity and resistance to fluoropyrimidines (FP).(20-24), In particular, five recent studies have consistently demonstrated an inverse correlation between the level of TS gene or protein expression measured in colorectal cancer metastases and the clinical response to either FUra or 5-fluorodeoxyuridine (FUdR).(20-24), Patients with low levels of TS expression in their metastases have indeed shown response rates that are three to ten times higher compared to those obtained in patients with high TS levels.(20-24)

The relationship between TS levels in primary colorectal cancer and response to chemotherapy for advanced disease has also been investigated for example a retrospective analysis of TS expression in locally-advanced rectal cancer treated with neoadjuvant 5-FU and radiation found 11 of 12 tumors with a marked response, negative for TS. However 5 of 13 non-responders also had no detectable TS.(25), But some study failed to demonstrate any relationship between TS expression and sensitivity to FP's.(26-28)

Patients with high TS levels in their metastatic tumors have almost no chances to obtain a clinical response with biochemically-modulated single agent FUra.(16), These patients clearly need combination chemotherapy as initial treatment for their disease and a regimen without FPs, like a combination of irinotecan and oxaliplatin, might be the optimal choice in this setting.(16)

The aims of this study were: (1) to assess the value of thymidylate synthase (TS) expression as a predictive factor in the efficacy of neoadjuvant chemoradiation with and without oxaliplatin in rectal cancer. (2) Verifying that whether oxaliplatin can improves probable poor outcome of TS positive rectal cancer?

MATERIALS AND METHODS

Patient selection and pretreatment evaluation

Criteria for patient inclusion were as follows: (1) histologically confirmed diagnosis of adenocarcinoma of the rectum, with the inferior margin of the tumor being no farther than 6 cm from the anal verge, (2) clinically staged locally advanced rectal cancer, (3) treatment with preoperative chemoradiation followed by surgical resection, and (4) availability of tissue samples of the diagnostic biopsy and tumor specimen for review and immunostaining.

From September 2003 to October 2005, 61 patients with locally advanced rectal adenocarcinoma were treated with preoperative chemoradiation in our institution that ultimately 50 patients had including criteria (4 patients refused treatment, 4 patients were inoperable and 3 patients were unresectable). There were 35 males and 15 females with a median age of 52 years (range: 28-80 years). Locally advanced rectal cancer was defined as tumor extension through the bowel wall (T3-T4) or with lymph node involvement (LN+). All patients were distant metastases-free at diagnosis. Local extension assessment was based on clinical and/or radiographic evaluations. Diagnostic studies consisted of colorectal endoscopy, abdominopelvic CT scan, chest X-ray, EUS and routine laboratory studies. Patients had histologically confirmed adenocarcinoma of the rectum, and the inferior margin of the tumor had to be located no farther than 6 cm from the anal verge. Patients were staged according to the American Joint Committee on Cancer Staging.

TS immunohistochemical assay (IHC): The detection of the TS antigen was carried out by the streptavidine-biotin-peroxidase technique (StreptABC, DAKO®). The primary antibody used was the TS-106 clone (Chemicon® code MAB-4130).

Briefly, the slides were first coated with a silano solution (APTS-Sigma® A3648) diluted to 4% in acetone. The formalin fixed, paraffin embedded tissues were cut into samples of 3 μm thickness after mechanical microtomy. The cuts were deparaffinized and prepared by successive passages through xylol and ethanol and submitted to antigenic recovery

pressure cooker heat, using a citrate buffer. Once the cuts were prepared, blocking of the peroxidase endogen with a 3% solution of hydrogen peroxide in methanol was carried out, followed by overnight incubation of the previously mentioned antibody, diluted in a pre-establish ratio of 1:100. The reactions were revealed with diaminobenzidine (DAB Sigma®) solution at 60 mg% and counter-colored with Harris hematoxyline (Merck®). The reactions were always accompanied by positive control in tissue known to be positive for the tested antibody and two negative controls. The first of these was carried out by the non-use of the primary antibody and the second by the removal of the secondary antibody during the steps of the reaction. All the slides were read by a single pathologist (F.A.S.), who was intentionally not informed of the clinical data.

Specimens were examined by light microscopy. All slides were assessed for TS expression by one trained histopathologists masked to tumor response to pelvic radiotherapy and the patients were categorized into TS (+) and TS (-) groups.

Preoperative chemoradiation and surgical modalities: Preoperative radiotherapy was delivered to the pelvis with CO 60. Clinical target volume included the tumor and the entire rectum, the anterior wall of the sacrum and the posterior wall of the prostate or vagina, and the following

lymph nodes: perirectal, presacral, hypogastric, actuator, and iliac lymph. The standard AP_PA fields to 45 Gy, and then 2 opposed laterals boost field up to 50/4, were used. All patients received conventional fractionation, 1.80 Gy/day, 5 fractions per week. All patients received simultaneous chemotherapy: 5-fluorouracil (5-FU), 300 mg/square meter i.v. 24 h infusional during radiotherapy on days 1-5 every week. Thirty of patients received oxaliplatin 50-60 mg/square meter weekly during radiotherapy. Patients were restaged by physical examination, pelvic CT, between 4 and 6 weeks later, then they referred to one surgeon that was expert in GI cancer surgery. The total duration of follow up was 12 weeks. Subsequently pathologic response evaluated in post surgical specimen and graded according to method described by Mandard *et al.*(51), in 1994 for the assessment of pathologic response after neoadjuvant chemoradiation in esophageal cancer on a scale from 1-5 based on the presence of residual tumor cells and the extent of fibrosis. Grade 1 that is defined as the absence of residual tumor and fibrosis extending through the different layers of the rectal wall we considered as complete pathologic response. Grade 2-4 that is characterized by the presence of various amounts of residual tumor cells, we considered as partial response. Grade 5 that is defined as of any tumor regression we considered as no response.

Statistical analysis: Possible associations between TS immunoexpression and clinical/histopathologic characteristics were determined using the chi-square test. Data analyses were performed with the SPSS statistical software package (SPSS, Inc., Chicago, IL). The significance level (α level) was p = 0.05 in all statistical analyses used in this study.

RESULTS

50 patients had including criteria. A total of 23 of 50 tumors showed TS positive status at biopsy (46%). Overall 36 patients (72%) achieved pathologic response (40% complete and 32% partial) that was significantly better in the TS (-) group than in the TS (+) group (85.1 vs 56.5%, p=0.024) (table 1). There is no significant difference according to age and sex by t-test also oxaliplatin consumption (p=0.643) between TS (-) and TS (+) groups.

Table 1. Clinicopathological response according to TS status

	Total		TS-		TS+	
	No.	**%**	**No.**	**%**	**No.**	**%**
	50	100	27	54	23	46
Treatment Type						
Rt-Ct (-Ox)	20	40	10	37	10	43
Rt-Ct (+Ox)	30	60	17	63	13	57
Pathologic Response						
Negative	14	28	4	15	10	43
Positive	36	72	23	85	13	57*
Sphincter Preserving						
Negative	14	28	6	22	8	35
Positive	36	72	21	78	15	65
Gender						
Male	35	70	18	66	17	73
Female	15	30	9	33	6	27
Pathological stage						
T3	28	56	15	55	13	56
T4	22	44	11	45	11	44
LN-	16	32	9	33	7	30
LN+	34	68	18	67	16	70

* p = 0.024

Among OX (+) patients 26 of 30 achieved pathologic response (9 partial and 17 complete response) but among OX (-) 10 of 20 achieved pathologic response (7 partial and 3 complete response) that was significantly better in the OX(+) group than in the OX(-) group (86.6 vs 50%, p=0.005) (table 2). Not only OX (+) group had higher response rate but also the majority (17

Table 2. Clinicopathological response as a result of whether oxaliplatin was carried out or not.

	Total		OX-		OX+	
	No.	%	No.	%	No.	%
	50	100	20	40	30	60
Pathologic Response						
No	14	28	10	50	4	13
Yes	36	72	10	50	26	87
Sphincter Preserving						
No	14	28	8	40	6	20
Yes	36	72	12	60	24	80
Gender						
Male	35	70	14	70	21	70
Female	15	30	6	30	9	30
Pathological stage						
T3	27	54	7	35	20	66
T4	23	46	13	65	10	44
LN-	15	30	7	35	8	26
LN+	35	70	13	65	22	73

of 26) of them had CR (65.4%) compare to OX(-) responsive patients that only 3 of 10 achieved CR (30%).

Among TS (-) patients there was no difference in pathologic response (88.2 vs 80%, p=0.561) or sphincter preservation (76.4 vs 80%, p= 0.831) as a result of whether oxaliplatin was carried out or not (table 3). But among the TS (+) patients there was a significant gain in pathologic response (84.6 vs 20 %, p=0.002) and sphincter preservation (84.6 vs 40 %, p= 0.026) in favor of oxaliplatin group.

DISCUSSION

Multiple recent studies, have demonstrated that the level of TS protein expression measured immunohistochemically in colorectal cancer metastases is significantly correlated with the clinical response to biochemically modulated FUra (fluoropyrimidines).(20-24), In one of this trials Aschele et al. have examined TS quantitation with immunohistochemical method ov paraffin. Sections of metastatic colorectal from 48 patients homogeneously treated with bolus and continuous infusion FUra and schedule specific biochemical modulators.(21), In this study, twenty- one of 48 patients (44%) showed high TS based on a visual grading scale for the intensity of staining and response to chemotherapy was found to be inversely correlated with the level of TS Protein expression. The combined complete response (CR)+ partial response (PR) rate was 67% and 24% in patients with low and high levels of TS expression, respectively, (p=0.003). More importantly, patients with low TS survived longer

Table 3. Comparison of Clinicopathological response as a result of whether oxaliplatin was carried out or not in TS(+) and TS(-) group.

	TS(+) Group						TS(-) Group					
	Total		OX-		OX+		Total		OX-		OX+	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	23	100	10	43	13	57	27	100	10	37	17	63
Pathologic response												
No	10	43	8	80	2	15	4	15	2	20	2	12
Yes	13	57	2	20	11	85	23	85	8	80	15	88
Sphincter preserving												
No	8	35	6	60	2	15	6	22	2	20	4	24
Yes	15	65	4	40	11	85	21	78	8	80	13	76

with a median survival time of 18.4 month compared to 15.4 month in patients with high TS (p=0.02).

Overall, these five studies consistently demonstrated an inverse correlation between the level of TS expression (either gene or protein expression) measured in colorectal cancer metastases and the clinical response to FPs.(16), In particular, patients with low level of TS expression in their colorectal metastases have shown response rates to FP based chemotherapy that are three to ten times higher compared to those obtained in patients with high TS levels.(16)

The relationship between TS levels in primary colorectal cancer and response to chemotherapy for advanced disease has also been investigated. For example Okonkwo *et al.* in a retrospective analysis of TS expression in locally-advanced rectal cancer treated with neoadjuvant 5-FU and radiation found 11 of 12 tumors with a marked response, negative for TS. However 5 of 13 non-responders also had no detectable TS.(25)

But some studies failed to demonstrate any relationship between TS expression and sensitivity to FP's. In particular, a recent work on a panel of 60 human cancer cell lines from the NCI's Anticancer Drug Screen failed to demonstrate a correlation between TS levels and sensitivity to either FUra o FUdR.(28), This last negative study is particularly important because of its quality and size.

Innate FP resistance seems thus to be less dependent on TS alterations as compared to the pattern previously described for acquired resistance. TS levels were in fact associated with sensitivity to FP's in approximately two thirds of the studies on multitumor panels.(16), Despite these limitations, it is remarkable that in all of these studies the level of response observed among patients with low TS level has been twice higher than that observed with the same chemotherapy regimens in unselected patient populations. Also more importantly, the level of response observed among patients with low TS levels treated with FP-monotherapy compares very favorably to that obtained with more toxic and more expensive combination regimens including new agents like irinotecan and oxaliplatin.(16)

In our study a total of 23 of 50 tumors showed TS positive status at biopsy (46%). Overall 36 patients (72%) achieved pathologic response (40% complete and 32% partial) that was significantly better in the TS(-) group than in the TS(+) group (85.1 vs 56.5%, p= 0.024).

As previously mentioned neither TS immunoreactivity nor the level of TS mRNA expression could fully predict for the clinical response to FUra-based chemotherapy. In particular, while high TS levels effectively predicted resistance to FUra, low TS did not necessarily result in a clinical response.(16), In particular, a proportion of patients ranging from 35% ± 50% fails to achieve a clinical response in spite of favourable (low) TS levels(in our study nearly 15%). These patients may have other mechanisms of resistance that the favourable condition of low TS is insufficient to overcome. As previously discussed, baseline pretreatment TS levels represents in fact only one of multiple determinants of TS inhibition.(32-35), In addition, both molecular factors modulating the pathways of cellular death down stream of TS inhibition (36-38) and clinical variables generally associated with the poor response to chemotherapy in advanced colorectal cancer (39,40) may mitigate the association of low TS level with a good clinical outcome. Several study indicate that combining more than one independent determinant of response such as dihydropyrimidine dehydrogenase (DPD) and the nucleoside cleavage enzyme thymidine phosphorilase (TP), that were found to be significantly associated with response to FUra-based chemotherapy (41,42) can increase the predictive power for clinical outcome.

Oxaliplatin is a third-generation platinum derivative, which, when combined with fluorouracil and leucovorin, is among the most effective chemotherapies for metastatic colorectal cancer.(9,43,44,45), so that can double the response rate and prolonged progression-free survival among patients with metastatic colorectal cancer.(45), More recently some phase II trials have demonstrated the feasibility and efficacy of combining oxaliplatin with FUra and RT in neoadjuvant setting for treatment of locally advanced rectal cancer.(12-15), Aschele et al.

showed 21 of 25 patients with recurrent or locally advanced (c T3-4 and/or N+) adenocarcinoma of the mid-low rectum were treated with OXA/FUra concurrent to preoperative pelvic radiotherapy had the tumor down staging with seven (28%) pathological complete response and 12 (48%) residual tumor limited to pT1-2N0.(12)

In our study overall 36 patients (72%) achieved pathologic response (40% complete and 32% partial). Among OX(+) patients 26 of 30 achieved pathologic response (9 partial and 17 complete response) but among OX(-) 10 of 20 achieved pathologic response (7 partial and 3 complete response) that was significantly better in the OX(+) group than in the OX(-) group (86.6 vs 50%, p= 0.005). Not only OX (+) group had higher response rate but also the majority (17 of 26) of them were CR (65.4%) compare to OX(-) responsive patients that only 3 of 10 achieved CR (30%). Like Aschele et al. trial the majority of our patients had the tumor down staging but CR rate in our study was higher. On the other hand we compare OX(+) patients with OX(-) patients that showed advantage of our study. Meanwhile, we reported the rate of sphincter preservation that had not reported in other trials.

Ultimately most important part of our study and main aim of it were to determine whether oxaliplatin can improve poor outcome of TS (+) patients undergoing neoadjuvant chemoradiation for locally advanced rectal cancer. In our knowledge this is the first trial that addresses this issue. In our study among TS (-) patients there was no difference in pathologic response (88.2 vs 80%, p= 0.561) or sphincter preservation (76.4 vs 80%, p= 0.831) as a result of whether oxaliplatin was carried out or not. But among the TS (+) patients there was a significant gain in pathologic response (84.6 vs. 20 %, p =0.002) and sphincter preservation (84.6 vs. 40 %, p = 0.026) in favor of oxaliplatin group.

Aschele *et al.* in their review that were published in 2002 indicated that patients with high TS levels in their metastatic tumors have almost no chances to obtain a clinical response with biochemically-modulated single agent FUra.(16), These patients clearly need combination chemotherapy as initial treatment for their disease and a regimen without FPs, like a combination of irinotecan and oxaliplatin, might be the optimal choice in this setting.

We can concluded based on this study in locally advanced recta cancer like metastatic rectal cancer patients with high TS levels clearly need combination chemotherapy as initial treatment for their disease and a regimen like a combination of 5FU and oxaliplatin, might be the optimal choice in this setting because the addition of oxaliplatin can improve pathologic response from 20% in 5FU containing chemoradiation group to 84.6% in 5FU-Oaliplatin containing chemoradiation group. On the other hand, Aschele *et al.* emphasized that patients with low TS may derive a similar benefit with less toxicity from FUra monotherapy compared to more toxic combination regimens. A sequential treatment program consisting of FUra-based chemotherapy up-front followed by irinotecan and/or oxaliplatin at the moment of progression might therefore be considered for this group of patients. Along Aschele conclusions we can show in TS (-) patients there is no difference in pathologic response (88.2 vs 80%, p = 0.561) or sphincter preservation (76.4 vs 80%, p = 0.831) as a result of whether oxaliplatin was carried out or not thus we can not advise oxaliplatin in this group of patients particularly this issue has acquired an even greater importance following the recent report of severe toxicity and multiple toxic deaths with combination chemotherapy for colorectal cancer.(46)

Clinical evaluation in prospective clinical trials is necessary to validate this tailored approach to the medical treatment of advanced colorectal cancer before it can be introduced in the clinical practice. Meanwhile, efforts should be made to improve the ability to predict for response and resistance.

Ultimately there is some limitation in our study. This study is not performed according to all criteria of a randomized trial and a study with the same number of subjects in two group is suggested.

CONCLUSION

Our study indicate that oxaliplatin can improves poor outcome of TS positive rectal cancer and TS

expression may be used for selecting patients for oxaliplatin containing neoadjuvant chemoradiation protocols that can have major role in the tumor down staging and preservation of sphincter and ultimately better quality of life.

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