Raltitrexed-induced hepatotoxicity: multivariate analysis of predictive factors

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Raltitrexed (Tomudex®; TOM) hepatotoxicity is usually characterized by a transient and self-limiting increase in transaminase levels. How this may condition daily clinical practice is still unclear. The aim of this study was to investigate predictive factors of TOM hepatotoxicity. In total, 130 patients were treated at two medical oncology institutions with TOM (3 mg/m^2) (52 patients) or TOM plus oxaliplatin (TOMOX) (100 mg/m^2 day 1 or 70 mg/m^2 day 1, 8) (78 patients). A multinomial logistic regression (adjusted for multilevel data) was performed (on all administered chemotherapy courses) to assess the dependence of hepatic toxicity on a set of clinical factors correlated with patient, disease and treatment characteristics. Creatinine clearance was calculated by the Cockcroft formula before each chemotherapy course. Most of the patients presented colorectal cancer (95%) and metastatic disease (93%). Out of the 130 patients, 41 were aged 70 or more, while 119 (91.5%) had a good performance status (PS) (ECOG 0 or 1). Before chemotherapy, liver metastases were present in 78 (60%) patients and elevated transaminase in 25 (19%). A total of 584 courses were administered (252 TOM and 332 TOMOX). National Cancer Institute Common Toxicity Criteria grade 1/2 and 3/4 transaminase toxicity was observed in 62 and 20% of patients, respectively. To control transaminase increase, glutathione (GSH) or ademethionine (SAMe) was administered in 96 and 129 cycles, respectively. Hepatotoxicity conditioned delays (a week or more) in 60 (10%) chemotherapy cycles and was the reason for the discontinuation of chemotherapy in eight (6%) patients. Among the factors evaluated with multivariate analysis, sex, age, PS, creatinine clearance, previous chemotherapy treatment, presence of liver metastases and oncology centre were not significantly associated with TOM hepatotoxicity. Elevated baseline transaminase levels ($p = 0.001$), number of chemotherapy cycles ($p < 0.001$), TOM cumulative dose ($p = 0.018$), unprolonged intervals between courses ($p < 0.001$) and TOMOX regimen ($p < 0.001$) emerged as factors predictive of hepatotoxicity. In the same analysis, GSH ($p < 0.001$) and SAMe ($p < 0.001$) were hepatoprotective agents. This study confirmed TOM-based hepatotoxicity as a clinical relevant side-effect and a major factor for treatment delays or discontinuation. Predictive and protective factors listed above could assist the management of this toxicity that has probably been underestimated until now. *Anti-Cancer Drugs* 14:533–541

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Introduction

Raltitrexed (Tomudex®; TOM) is a quinazoline antifolate that specifically inhibits thymidylate synthase. It is extensively polyglutamated by intracellular folyl polyglutamate synthase to metabolites that are up to 100 times more potent than the parent compound at inhibiting thymidylate synthase [1]. Tomudex entered clinical development in 1991, and since the publication of the results of phase I dose-finding studies, an extensive phase II and III clinical study programme has been undertaken to study its clinical efficacy and tolerability [2]. Several clinical trials have investigated the activity of this drug in patients with a wide spectrum of malignancies, but the largest reported experience has been for first-line treatment of patients with advanced colorectal carcinoma [3,4]. Data from four phase III studies including about 1000 patients with advanced colorectal cancer treated with raltitrexed, showed comparable objective response rates between raltitrexed and bolus or infusional 5-fluorouracil (5-FU)/leucovorin (LV) [5–8]. One of these studies showed a survival advantage for patients treated with bolus 5-FU/LV [6].

The treatment with raltitrexed was well tolerated by the majority of patients. The drug has been associated with a lower rate of leukopenia and mucositis when compared to

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bolus 5-FU/LV [5–7], but raltitrexed resulted in a significant higher incidence of neutropenia, thrombocytopenia, diarrhea and lethargy compared to infusional 5-FU/LV [8]. Raltitrexed deaths appeared to be due to a combination of diarrhea and myelosuppression with infection [9]; therefore, appropriate delays and dose reduction have been suggested after hematological and gastrointestinal toxicity, and depending on creatinine clearance [3,4]. Tomudex is currently approved as first-line therapy for patients with advanced colorectal cancer in over 40 countries worldwide.

Recently the combination of raltitrexed and the platinum derivative oxaliplatin [10] as first-line treatment demonstrated high activity in terms of response rate and survival times in phase II trials [11–14]. Although these two drugs demonstrated different toxicity profiles, hematologic and hepatic toxicity seems to be higher with the combination compared to raltitrexed alone.

Transient elevation in hepatic transaminases has been reported as the most common side-effect observed in raltitrexed-based chemotherapy [9]. No delay or dose reduction criteria have yet been contemplated for this toxicity [3,4], and it has not been clarified whether there are any common clinical factors, such as age, sex, performance status (PS), disease in the liver, etc., capable of predicting transaminitis due to raltitrexed-based treatment. Nevertheless, although transaminitis after raltitrexed-based chemotherapy is generally considered to be asymptomatic and self-limiting, some cases of fatal liver toxicity have been reported [15].

N-acetylcisteine, a precursor of glutathione (GSH), and other sulfhydril donors are specific antidotes for hepatotoxicity, effective, for example, in the treatment of paracetamol-induced liver failure [16]. Also *S*-adenosylmethionine (SAMe), providing cysteine for the production of endogenous GSH, has been demonstrated to be useful in liver impairment [17,18]. Conversely, the role of GSH and SAMe to prevent chemotherapy-induced hepatic toxicity is not yet completely clear.

Therefore, the aim of this study was to analyze how hepatic toxicity induced by raltitrexed-based chemotherapy may condition dose reductions, cycle delays and treatment discontinuations. Moreover, we evaluated clinical factors capable of predicting transaminase elevation and the role of hepatoprotectors in patients with metastatic gastrointestinal cancer treated under routine clinical conditions.

Patients and methods

Patient selection

Patients for this study were identified from a prospectively acquired database of patients who had been administered raltitrexed as single-agent or in combination therapy with oxaliplatin from October 1998 to December 2001. All patients had histologically proven gastrointestinal tumor. One hundred and thirty patients were treated in this period at the Medical Oncology of Ancona (79 patients) and at the 'Campus Biomedico' Medical Oncology of Rome (51 patients), and were evaluated as suitable for analysis. Forty-nine (37.7%) patients receiving raltitrexed in combination chemotherapy were enrolled in clinical trials previously reported [12,19]. Written informed consent was obtained from each patient before treatment. Patients' PS was graded according to the Eastern Cooperative Oncology Group (ECOG) scale.

Treatment plan

Patients were treated following these schedules:

- Raltitrexed as single agent 3 mg/m^2 i.v. over 15 min day 1 (cycles every 3 weeks) (52 patients).
- Raltitrexed (3 mg/m^2 i.v. day 1) followed by 2-h i.v. oxaliplatin 100 mg/m^2 day 1 (cycles every 3 weeks) (38 patients).
- Raltitrexed (3 mg/m^2 i.v. day 1) plus oxaliplatin 70 mg/m^2 i.v. days 1 and 8 (cycles every 3 weeks) (40 patients).

Chemotherapy was administered on an inpatient or outpatient basis according to institutional and/or patient preference. Prophylactic antiemetics were ondansetron 8 mg or granisetron 3 mg, plus dexamethasone 4 or 8 mg, all administered i.v. Creatinine clearance before raltitrexed administration was measured by the Cockcroft–Gault formula [20].

Before chemotherapy all patients had adequate bone marrow reserve [white blood cell (WBC) count $\geq$ 3000/mm^3, neutrophil count $\geq$ 1500/mm^3, platelet count $\geq$ 100 000/mm^3, and hemoglobin $\geq$ 9 g/dl) and normal hepatic excretory function (bilirubin $<$ 1.5 mg/dl).

Generally the chemotherapy cycle was delayed if the WBC count was $<$ 3000/mm^3, neutrophil count $<$ 1500/mm^3 and/or platelet count $<$ 100 000/mm^3. In patients with febrile or grade 4 neutropenia, severe thrombocytopenia, or severe non-hematologic toxicity, doses were reduced by 25–30% for subsequent cycles. In case of grade 4 non-hematologic toxicity treatment was discontinued. In heavily pretreated or poor PS patients a dose reduction was often planned from the first cycle.

Supportive care, including the administration of granulocyte colony stimulating factors, blood transfusions, erythropoietin, antibiotics, antiemetics and analgesics, was provided if deemed appropriate by physician. Oral SAMe (Samyr®, 200 or 400 mg tablets; Knoll Farmaceutici, Liscate, Italy) or GSH (Tad 600®, 600 mg phial;

Biomedica Foscama, Ferentino, Italy) were supplemented in case of hepatic toxicity (transaminase elevation).

Evaluation of toxicity

Toxicity was graded according to National Cancer Institute (NCI) Common Toxicity Criteria (CTC) [21], and was assessed before each chemotherapy administration by physical examination, direct questioning and measurement of hematologic and biochemical parameters.

Hepatic toxicity was evaluated by testing biochemical parameters such as transaminase SGPT or alanine aminotransferase (ALT), transaminase SGOT or aspartate aminotransferase (AST), alkaline phosphatase (ALK), γ-glutamyl transpeptidase (GGT), and total bilirubinemia. According to NCI scale, an impaired liver function with AST, ALT and ALK abnormal with respect to upper laboratory normal (ULN) was graded as follows: grade $1 = \leq 2.5 \times$ ULN, grade $2 = 2.6–5.0 \times$ ULN, grade $3 = 5.1–20.0 \times$ ULN and grade $4 = > 20.0 \times$ ULN. The enzyme GGT, like ALK, increases with liver damage and particularly for biliary tree diseases. Furthermore, in the presence of biliary damage, GGT usually shows higher increases compared to ALK. Although NCI criteria do not list GGT in the evaluation of hepatic toxicity, we considered it together with ALK (both are cholestasis indexes) and we graded GGT abnormal values as for ALK.

The NCI scale [21] does not contemplate bilirubin grade 1 toxicity (grade $2 = < 1.5 \times$ ULN, grade $3 = 1.5–3.0 \times$ ULN and grade $4 = > 3.0 \times$ ULN). For patients with liver metastases the increase of biochemical hepatic markers was considered related to raltitrexed-based chemotherapy only if the correlation with hepatic disease was definitely excluded.

Fatigue or asthenia was graded as mild, moderate and severe.

Statistical methods

The aim of this study was to identify risk factors for hepatotoxicity caused by raltitrexed chemotherapy. Single cycles of therapy were considered as sample units. Then, a proportional-odds model, adjusted for multilevel data, was developed in order to investigate the dependence of the toxicity score on a set of explanatory variables. These variables were chosen among clinical factors related to: characteristics of the patients (age, sex and PS), renal and hepatic function (creatinine clearance and baseline transaminase value), characteristics of disease (liver metastases), characteristics of treatment (previous treatments, regimen, number of cycles, raltitrexed cumulative dose, interval between cycles and institution) and use of hepatoprotectors (GSH and SAMe). The significance of risk factors was assessed by likelihood ratio tests, i.e. comparing the difference in 2-log likelihoods between the complete model and a reduced model obtained by the elimination of each single potential risk factor. The variables predicting a reduction or an increase of hepatotoxicity risk had been obtained by coefficients of the single variables of the model. A significance level was fixed at 0.05. Median-test was applied to evaluate differences among medians of continuous distributions of variables. A significance level was again fixed at 0.05.

Results

Patients and disease characteristics

One hundred and thirty patients were analyzed. The characteristics of the patients and the disease are listed in Table 1. Most patients were male (60.8%), with a median age of 65 years (range 34–78), and 41 (31.5%) patients were aged 70 or more. Most patients (91.5%) had a good performance status (ECOG 0 or 1).

Eighty-six and 38 patients had primary tumor located in the colon and rectum, respectively. Six patients had a gastrointestinal primary tumor of non-colorectal origin. One hundred and twenty-one (93.1%) patients had metastatic disease—most of them (53.1%) at two or more sites, with liver, distant nodes and lung as the

Table 1 Patient characteristics

Characteristic	No. of patients	%
Total	130	100.0
Sex		
female	51	39.2
male	79	60.8
Age (years)		
median	65	
range	34–78	
ECOG PS		
0	69	53.0
1	50	38.5
2	11	8.5
Primary tumor site		
colon	86	66.1
rectum	38	29.2
others[a]	6	4.6
No. metastatic sites of disease		
0 (adjuvant)	9	6.9
1	52	40.0
2	39	30.0
≥ 3	30	23.1
Metastatic sites		
liver	78/130	60.0
distant lymph nodes	51/130	39.2
lung	41/130	31.5
peritoneum/omentum	24/130	18.5
locoregional relapse	22/130	16.9
others[b]	15/130	11.5
Previous lines of chemotherapy (adjuvant or for metastatic disease)		
0	79	60.8
1	34	26.1
≥ 2	17	13.1
Baseline elevated transaminases	25	19.2

[a]Small intestine, ampulla of Vater, stomach, gastrointestinal unknown origin.
[b]Bone, central nervous system, spleen, adrenal, pancreas.

Table 2 Raltitrexed treatment characteristics

Characteristic	Raltitrexed alone	Raltitrexed/oxaliplatin	Total
No. of administered cycles			
total	252 (43.2%)	332 (56.8%)	584
median per patient (range)	5 (1–12)	4 (1–9)	4 (1–12)
Raltitrexed weekly doseintensity (mg/m^2)			
mean	0.90	0.80	0.84
range	0.58–1.05	0.34–1.00	0.34–1.05
Raltitrexed cumulative dose (mg/m^2)			
mean	14.0	11.2	12.3
range	3.0–36.0	1.5–27.0	1.5–36.0
Creatinine clearance before each chemotherapy cycle[a]			
median (range) (ml/min)	67 (36–115)	74 (33–181)	72 (33–181)
<65 (ml/min)	101 (40.1%)	85 (25.6%)	186 (31.8%)
unknown	4 (1.6%)	13 (3.9%)	17 (2.9%)

[a]Calculated by the Cockcroft and Gault formula.

commonest metastatic sites (60.0, 39.2 and 31.5% of patients, respectively).

Seventy-nine (60.8%) patients received raltitrexed as a single-agent (TOM) or raltitrexed plus oxaliplatin (TOMOX) as first-line chemotherapy. Transaminases were elevated at baseline in 25 patients, without significant differences between those treated with TOM (seven patients) or TOMOX (18 patients) ($p = 0.37$).

Raltitrexed treatment characteristics

As reported in Table 2, TOM and TOMOX were administered to 52 (40.0%) and 78 (60.0%) patients, respectively. A total number of 252 chemotherapy cycles of TOM and 332 of TOMOX were delivered to patients, with a median number of cycles administered per patient of 5 (range 1–12) and 4 (range 1–9), respectively.

Although mean weekly raltitrexed dose intensity and cumulative dose were higher when the drug was employed as a single-agent compared with the combination schedule, no significance was reached: 0.9 versus 0.8 mg/m^2/week and 14.0 versus 11.2 mg/m^2 ($p = 0.57$ and $p = 0.18$, respectively).

No patients were administered raltitrexed-based chemotherapy with creatinine clearance below 25 ml/min. Raltitrexed was administered to patients with a creatinine clearance value of 55–65 and 25–54 ml/min in 154 (26.4%) and 50 (8.6%) cycles, respectively. Nevertheless an appropriate dose reduction as recommended by the Tomudex guidepaper was applied in 64 of 204 (31.4%) cycles. A dose reduction of the drug was less likely to be administered to patients treated with raltitrexed as a single agent (TOM 103 cycles versus TOMOX 37 cycles, $p < 0.001$). If, instead, serum creatinine was considered instead of clearance, raltitrexed was administered when it was higher than the normal upper limit (1.4 mg/dl) only in seven (1.2%) cycles.

Table 3 Treatment delays or discontinuation

Reasons for delays or stopping prematurely	Delays (cycles)	Discontinuation (patients)
Hepatotoxicity	60 (10.3%)	8 (6.1%)
Gastrointestinal toxicity[a]	5 (0.8%)	5 (3.8%)
Hematological toxicity[b]	13 (2.2%)	3 (2.3%)
Other toxicities[c]	11 (1.9%)	3 (2.3%)
Patient's choice	5 (0.8%)	11 (8.5%)
Physician's decision	7 (1.2%)	1 (0.8%)
Concomitant illness	1 (0.2%)	3 (2.3%)
Radiological re-evaluation	18 (3.1%)	–
Progressive disease	–	34 (26.1%)
Death	–	2 (1.5%)
Total	584	130

[a]Diarrhea, mucositis.
[b]Leukopenia, neutropenia, thrombocytopenia.
[c]Infection, asthenia, cardiovascular, neurological, toxic death.

In patients who developed hepatotoxicity we tended to delay cycles to maintain a full dose of the drug in subsequent administration; thus only in 10 (1.7%) cycles was the dose of raltitrexed reduced as a consequence of transaminase toxicity manifested during the previous cycle.

Chemotherapy delays were observed in 118 (20.2%) cycles, with a median delay of 7 days (range 1–38 days). The commonest reason for delay was chemotherapy side-effects (87 of 118 cycles), namely hepatic toxicity (Table 3). In 18 cases (15.2%) instrumental re-assessment of disease led to delay the subsequent cycle.

Sixty (46.2%) patients completed the 'treatment period' (at least 6 cycles). The remaining population, as shown in Table 3, stopped treatment prematurely, mainly because of progressive disease (34 patients), unacceptable toxicity (19 patients, eight of which for severe hepatic impairment), refusal to go ahead with treatment

(11 patients) or worsening of concomitant illnesses (three patients).

Overall toxicity

In general, treatment was well tolerated and NCI-CTC recorded toxicities were mild (Table 4). Few cases of grade 3/4 leuko-neutropenia, anemia or thrombocytopenia were observed. There were only two cases of febrile neutropenia, resolved with antibiotic therapy and G-CSF. No patients required red blood or platelet transfusions. The majority of non-hematologic toxicities was again grade 1/2 and consisted mainly of neuropathies, nausea/vomiting, diarrhea, constipation and asthenia.

Combination chemotherapy was significantly associated with more sensory neuropathies ($p < 0.001$), nausea ($p < 0.03$) and asthenia ($p < 0.005$) compared with raltitrexed alone, but no significant differences were observed between TOM and TOMOX for severe (grade 3/4) toxicities.

Skin toxicity (rash) was observed in 10 patients, eight of whom had been treated with combination chemotherapy regimen. Mild infections and hypersensitivity reactions were recorded in five and two patients, respectively, all treated with the combination regimen. Transient atrial fibrillation arose in two patients, probably correlated to chemotherapy (raltitrexed alone in one patient and the combination in the other). There was one (0.8%) treatment-related death in a patient with a high baseline creatinine value (more than 1.4 mg/dl), and a calculated creatinine clearance before each chemotherapy course between 34 and 46 ml/min. She received five cycles of TOMOX at 75% of the planned dose. Treatment was well tolerated until the fifth cycle when she had an early worsening in renal impairment (creatinine 4.0 mg/dl) with a combination of hematologic (grade 4 neutropenia and thrombocytopenia) and gastrointestinal (grade 4 mucositis, grade 3 diarrhea, grade 3 vomiting and grade 3 bilirubin) toxic effects that led her to death.

Hepatic toxicity

An elevation of transaminases was commonly observed during chemotherapy (50% of cycles) and it was severe in 26 (20%) patients (Table 5). An impaired liver function with abnormal ALK/GGT or bilirubin was less frequent. A severe elevation of these indices was recorded in four and three patients, respectively (Table 5). Combination chemotherapy was more likely to cause transaminase ($p < 0.001$), ALK/GGT ($p < 0.001$) and bilirubin ($p < 0.02$) toxicity compared with raltitrexed alone, but severe toxicity in patients treated with TOMOX was significantly higher only for transaminases ($p < 0.001$).

All 26 patients who developed severe hepatotoxicity, and some patients with moderate transaminase elevation, received hepatoprotectors before and during subsequent chemotherapy courses. Oral SAMe was supplemented in 129 cycles (122 TOMOX and 7 TOM, $p < 0.01$) to 36 patients at a median dose of 800 mg/day (range 200–1600 mg) until subsequent cycle. Glutathione (GSH) was administered orally or i.m. in 96 cycles (64 TOMOX and 32 TOM, $p < 0.05$) to 30 patients at a median dose of 600 mg/day (range 600–2400) for a median duration of 7 days (range 2–21 days) in each cycle.

Risk factors for hepatic toxicity

A proportional-odds model, adjusted for multilevel data, was used on our cohort to investigate the simultaneous effects in predicting the hepatic toxicity evaluated by

Table 4 Toxicities (excluded hepatic) by patient (NCI-CTC scale)

Side-effect	Raltitrexed ($n=52$)				Raltitrexed/oxaliplatin ($n=78$)			
	Grade 1/2		Grade 3/4		Grade 1/2		Grade 3/4	
	Patients	%	Patients	%	Patients	%	Patients	%
Hematologic								
leukopenia	10	19.2	2	3.8	19	23.1	2	2.6
neutropenia	4	7.7	3[a]	5.8	11	14.1	5[a]	6.4
anemia	14	26.9	1	1.9	20	25.6	2	2.6
thrombocytopenia	1	1.9	1	1.9	7	9.0	3	3.8
Gastrointestinal								
nausea	21	40.4	1	1.9	43	55.7	5	6.4
vomiting	13	25.0	–	–	23	29.5	3	3.8
mucositis	5	9.6	–	–	13	16.7	2	2.6
diarrhea	8	15.4	4	7.7	18	23.1	7	9.0
Neurological								
sensory	–	–	–	–	49	62.8	1	1.3
constipation	11	21.1	–	–	13	16.7	2	2.6
Other symptoms								
asthenia	2	3.8	–	–	17	21.8	2	2.6
skin	2	3.8	–	–	7	9.0	1	1.3
fever	3	5.8	–	–	7	9.0	–	–
various[b]	1	1.9	–	–	9	11.5	1	1.3

[a]Febrile neutropenia (neutrophil count $<500/mm^3$ and fever $>38.5^{\circ}C$) in one patient.
[b]Infection, cardiac, renal, hypersensitivity, anorexia.

Table 5 Hepatotoxicity by patient and cycle (NCI-CTC scale) [*N* (%)]

	Total		Raltitrexed alone		Raltitrexed/oxaliplatin	
	Patients ($n=130$)	Cycles ($n=584$)	Patients ($n=52$)	Cycles ($n=252$)	Patients ($n=78$)	Cycles ($n=332$)
Transaminase						
grade 1/2	81 (62.3)	292 (50.0)	32 (61.5)	89 (35.3)	49 (62.8)	203 (61.1)
grade 3/4	26 (20.0)	33 (5.6)	3 (5.8)	4 (1.6)	23 (29.5)	29 (8.7)
ALK/GGT						
grade 1/2	49 (37.7)	113 (19.3)	10 (19.2)	23 (9.1)	39 (50.0)	90 (27.1)
grade 3/4	4 (3.1)	5 (0.8)	2 (3.8)	2 (0.8)	2 (2.6)	3 (0.9)
Bilirubin						
grade 2	6 (4.6)	8 (1.4)	1 (1.9)	1 (0.4)	5 (6.4)	7 (2.1)
grade 3/4	3 (2.3)	4 (0.7)	–	–	3 (3.8)	4 (1.2)

Table 6 Risk factors for raltitrexed GOT/GPT toxicity (all grades by cycle)

Risk factors	−2 log likelihood of reduced model	χ^2	d.f.	*p* value
Sex (male versus female)	1049.876	4.741	3	0.192
Age (continuous variable)	1046.252	1.116	3	0.773
Performance status (0 versus 1 versus 2)	1055.192	10.056	6	0.122
Creatinine clearance (continuous variable)	1048.725	3.589	3	0.309
Baseline transaminase value (normal versus elevated)	1062.417	17.282	3	**0.001**
Liver metastases (yes versus no)	1047.706	2.570	3	0.463
Previous chemotherapy before raltitrexed (yes versus no)	1045.461	0.325	3	0.995
No. cycles (continuous variable)	1086.068	40.932	3	**<0.001**
Raltitrexed cumulative dose (continuous variable)	1055.235	10.100	3	**0.018**
Interval between raltitrexed cycles (continuous variable)	1079.045	33.909	3	**<0.001**
Treatment received (raltitrexed versus raltitrexed/oxaliplatin)	1067.432	22.296	3	**<0.001**
Center (Ancona versus Rome)	1057.210	6.118	3	0.106
GSH (yes versus no)	1063.315	18.179	3	**<0.001**
SAMe (yes versus no)	1067.871	22.735	3	**<0.001**

Bold shows significant *p* value <0.05.

GOT/GPT elevation (all grades considered). Factors related to patient (sex, age, PS, creatinine clearance and baseline transaminase level), disease (presence of liver metastases), raltitrexed-based treatment (previous chemotherapy treatments, number of cycles, raltitrexed cumulative dose, kind of regimen, interval between cycles and chemotherapy regimen) and supportive drugs (hepatoprotectors) were the variables included in the model (Table 6).

Factors that retained significance as risk factors included elevated baseline transaminase levels, high number of received chemotherapy cycles, high cumulative dose of raltitrexed administered, unprolonged interval between courses and TOMOX regimen. In the same analysis supplementation of both GSH and SAMe revealed their hepatoprotective action.

Discussion

The purpose of this study was to determine whether there were clinical parameters capable of predicting transaminase elevation induced by raltitrexed-based chemotherapy and whether hepatic toxicity may influence the treatment in patients with metastatic gastrointestinal cancer treated under routine clinical conditions.

In our population among the 52 patients who received TOM chemotherapy the toxicities usually reported with this drug, such as leuko-neutropenia, nausea/vomiting, diarrhea, hepatotoxicity and asthenia, were rarely severe. Grade 3/4 transaminase elevation was observed in 5.8% of TOM patients, similar to previously reported data [5–7]. For patients treated with TOMOX combination ($n = 78$) we again observed rates of hematological, gastrointestinal and oxaliplatin-related peripheral neuropathies, overlapping those already published [11–14].

The association of raltitrexed with oxaliplatin had demonstrated an *in vitro* additive effect [22]. It has been hypothesized that raltitrexed, binding TS, leads to the depletion of dTTP that may interfere with excision or mismatch repair systems. These represent the main repair mechanisms of DNA strand breaks induced by platinum derivative agents [4]. Moreover, the raltitrexed–oxaliplatin combination is attractive for the advantage of short infusion time avoiding i.v. infusion by central line as

in schedules with repeated and prolonged fluorouracil infusion [23]. In phase II trials, the raltitrexed and oxaliplatin regimen demonstrated high activity in terms of response rate, median time to progression and overall survival [11–14], a result that may be compared to that obtained with CPT-11/5-FU/LV [24–25] and L-OHP/5-FU/LV regimens [26].

As for hepatic toxicity, it is by now clear that oxaliplatin increases transaminitis caused by raltitrexed. Also, in combination with oxaliplatin, a dose of 3 mg/m^2 every 3 weeks has been suggested [27]. In the Cascinu *et al.* study [12] where oxaliplatin was administered at a dose of 100 mg/m^2, grade 3/4 transaminase elevation has been reported in 17% of patients. In those studies where patients were treated with oxaliplatin at 130 mg/m^2, severe hepatic toxicity was observed in 14–33% of the patients [11,13]. Our data seems to confirm these findings. In fact, patients treated in our series with the TOMOX regimen (median oxaliplatin dose 120 mg/m^2) developed transaminitis in 92% of cases and grade 3/4 in 29%. Therefore, as for sensory neuropathies, nausea and asthenia, we observed that hepatic toxicity (ALK/GGT, bilirubin and transaminase) was significantly more frequent with TOMOX compared with TOM. Moreover, multivariate analysis clearly confirmed that the combination chemotherapy with raltitrexed and oxaliplatin represented an independent risk factor for transaminase toxicity.

Proportional-odds regression also showed that factors related to the exposure to raltitrexed induce hepatic toxicity. Thus, the cumulative dose of raltitrexed administered, the number of cycles of chemotherapy received and an interval without delays between the cycles were independent factors for transaminase toxicity.

Raltitrexed is cleared from the body principally by renal excretion and its clearance follows a three-compartment elimination model with a terminal half-life of 10–22 h. The Tomudex guidepaper suggests a dose reduction of 50 or 25% if creatinine clearance is 25–54 or 55–65 ml/min respectively, and the interruption of raltitrexed administration if below 25 ml/min. In our population a calculated value of creatinine clearance (Cockcroft–Gault formula) [20] below to 65 ml/min was recorded before 35% of all cycles; however, in most of these cycles (68%), the recommended dose reduction was not applied. On the other hand, no patients had a severe reduction of creatinine clearance (below 25 ml/min) before raltitrexed chemotherapy and only in 7 cycles raltitrexed was administered with serum creatinine levels higher than the normal upper limit (1.4 mg/dl). Multivariate analysis did not find the creatinine clearance value as a prognostic factor for hepatotoxicity, whether it was calculated as a continuous or discrete (>65 versus ≤ 65 ml/min) variable. Nevertheless, although calculated creatinine clearance may not have a definite role in hepatotoxicity, it remains essential to evaluate renal function prior to and during the treatment in order to avoid other potential life-threatening side-effects [3,4].

If renal function did not seem to have prognostic significance for hepatotoxicity, the pre-existing elevated level of transaminases in our analysis conversely emerged as an independent factor significantly associated with a higher frequency of transaminitis. However, none of the patients with abnormal transaminases at baseline developed a life-threatening side-effect. This finding may extend and complete the general recommendation previously reported to avoid the use of raltitrexed in patients with hepatic impairment [3]: an elevated transaminase level before raltitrexed should suggest extreme caution, but it does not represent an absolute contraindication for the use of raltitrexed-based chemotherapy.

The hepatic toxicity of raltitrexed has generally been reported as transient and self-limiting [9], and it was considered a minor treatment-related problem in some studies where the occurrence of elevated transaminases levels was not collected at all [8]. In this context, no delay or dose reduction criteria have been contemplated for hepatic toxicity [3,4], and no influence of transaminitis has been evaluated in the clinical course of patients receiving raltitrexed-based chemotherapy, especially those treated with the raltitrexed and oxaliplatin combination in whom severe hepatic toxicity represents a major problem. According to our data, where more than 60% of patients were treated off-study, hepatotoxicity had a great clinical impact: it represented the most frequent side-effect cause of cycle delays and treatment discontinuation. One cycle out of 10 was delayed for hepatic toxicity and about one patient out of five (excluding those who progressed) prematurely stopped raltitrexed-based chemotherapy for the same reason.

Zalcberg reported the beginning of transaminase elevation during the second cycle of raltitrexed, with the highest peak during the third or fourth cycles and normalization with further cycles [9]. In our series, grade 3/4 transaminitis, recorded in 33 cycles, appeared in 70% of cases during the first or second cycle, and in the remaining 30% of cases between cycles 3 and 5. However, all the 26 patients who experienced severe transaminase toxicity were supplemented with hepatoprotectors and 11 (42%) discontinued treatment within the second cycle (eight for toxicity and three for disease progression). Therefore, according to our data, severe transaminase toxicity is usually observed between the second and the fourth cycle mainly because those patients who experienced toxicity are more likely to discontinue the

treatment early or to be administered hepatoprotectors that may reduce toxicity. On the basis of these data we could not exclude the development of hepatotoxicity in patients prolonging treatment beyond the fourth cycle.

Chemotherapy by the formation of reactive metabolites and free radicals might cause liver impairment sustained by functional and structural lesions, eventually resulting in cellular death and consequently in transaminases elevation [28]. GSH has been considered a specific antidote for hepatotoxicity as GSH levels are generally related to oxidative stress; moreover, the hepatic GSH system is specifically able to reduce oxygen-derived free radicals and reactive metabolites [16,29]. By stabilizing the cell membrane and providing cysteine for the production of endogenous GSH, SAMe has also been demonstrated to be active in the treatment of many types of liver injury [17,18,30]. We extensively employed both GSH and SAMe in the patients who experienced hepatic toxicity, with good compliance and without side-effects. Multivariate analysis demonstrated both GSH and SAMe as independent protective factors for transaminase elevation. Apparently both hepatoprotectors did not reduce activity of chemotherapy, although appropriate studies should be planned.

In conclusion, raltitrexed with its convenient administration schedule still represents a valid alternative to 5-FU and in association with oxaliplatin it demonstrated a very promising activity in advanced colorectal cancer. Nevertheless, apart from treatment efficacy, in this palliative setting what plays a relevant role is the toxicologic profile. Although some authors indicated some criteria for a more rational dosing scheme, in an effort to minimize raltitrexed toxicity, no guidelines have yet been given with regard to hepatic toxicity. Our study indicated raltitrexed-based hepatic toxicity as a relevant clinical side-effect and a major factor for treatment delays or discontinuation. Therefore the clinical parameters that we indicated as predictive together with protective factors could assist the management of this toxicity, which has so far been underestimated.

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