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Study of Nitrosourea Glycosyl Analogs—V. An oriented phase II Trial of RFCNU*

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Abstract—*Fifty-seven patients with digestive tract tumors or metastatic tumors of unknown origin were treated in a phase II trial with RFCNU [chloro-2-ethyl-1-ribofuranosyl-(isopropylidine-2'-3'-paranitrobenzoate-5')3-nitrosourea] administered on a two-day schedule. Doses were based on the results of a phase I trial, and ranged from* 300 *to* 350 mg/m^2. *When hematological tolerance was excellent, the dose was escalated to* 570 mg/m^2. *In cases of documented bone-marrow intolerance, we decreased the total dose to below* 300 mg *and even gave only* 150 mg/m^2 *to one patient. Courses were repeated every month. Three complete remissions (CR) and five partial regressions (PR) were achieved among* 57 *patients available for assessment of response. The overall rate of major responses (CR + PR) (PR being regressions superior to* 50%) *was* 14%, *with a median duration of* 9 months. *These major responses included* 4 *cases of liver metastases (including* 2 *CR) and* 3 *lung metastases of colorectal carcinoma (one CR). The responses were obtained at doses in excess of* 275 mg/m^2. *We also obtained* 30% *CR + PR in the case of a metastasis of unknown origin. This above evaluation is severe because it only reports major responses according to the WHO–EORTC–NCI recommendation. We also mention in complement* 12% *responses inferior to* 50% *in colorectal cancers, one in a stomach carcinoma, one in a hepatoma and* 15% *more in metastasis of unknown site primary tumors. The hematological toxicity was mild. It consisted of thrombocytopenia* 18%, *which was only dose-limiting in* 3 *patients* (6%) *previously treated with other nitrosoureas. Leukopenia with white cell counts of* 1500/mm^3 *was rarely observed. In* 32 *previously untreated patients, no correlation was found between hematologic toxicity and the number of cycles administered. Nausea and vomiting were noticed in* 14% *of patients. These results indicate that the efficacy of RFCNU for digestive tract tumors is at least comparable and probably slightly superior to that of other nitrosoureas. Adverse reactions, and mainly with hematological toxicity, were less marked that those observed with BCNU, CCNU or MeCCNU and do not appear to be cumulative.*

INTRODUCTION

SEVERAL nitrosourea (NU) analogs have undergone clinical evaluation. The phase II trial results of these drugs have been reviewed by Carter for BCNU [1], and Wasserman *et al.* for CCNU [2] and methylCCNU (MeCCNU) [3]. These three NUs have a broad spectrum of antitumor activity in human malignancies and have shown a significant therapeutic effectiveness in Hodgkin's disease (BCNU, CCNU) and in non-Hodgkin's lymphomas (BCNU and CCNU). They are the only class of drugs with consistent activity for CNS tumors [1]. In respect to digestive tract (DT) tumors, Moertel [4] reported an overall 17.5% response rate ['complete remissions (CR) + partial responses (PR)'] with MeCCNU, but the collected results in the literature suggest a somewhat lower expectation: the rates of CR + PR for BCNU are 12.5% in colorectal carcinomas, 0% in pancreatic cancers and 12% in the overall GI tumors [1]; for CCNU, a 10% response in colorectal cancer and in the overall GI neoplasias is described [2]; for MeCCNU a 11% response has been reported for phase II trials of the major GI tumor site [3].

Moreover, the use of most NUs in cancer

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chemotherapy has been limited by the delayed and cumulative bone marrow toxicity demonstrated mostly by the long-lasting and often severe thrombocytopenia which necessitates shortening the duration of treatment, and obliging to leave long intervals between two cycle administrations.

We performed a phase II study of a series of second generation NU analogs in the formula of which the cyclohexyl group of CCNU is replaced by a sugar molecule related to ribose, xylose or glucose [5, 6]. Our aim was to identify one or several analogs which were effective in DT neoplasias and which could be effective in tumors resistant to the first generation of NU analogs, without inducing some myeloid or platelet toxicity [7].

Among 37 analogs [8] produced for us by Montero *et al.* [5, 6] for testing against L1210 leukemia, only RFCNU (or (chloro-2-ethyl)1 - ribofuranosyl - isopropylidene - 2' - 3' - paranitrobenzoate-5')3-nitrosourea) (Fig. 1) and RPCNU (or (chloro-2-ethyl)-1-(ribopyranosyl triacetate-2'-3'-4')3-nitrosourea) gave a curve of dose–effect relationship presenting a plateau [9] called the 'maximally efficient dose range' (MEDR). This parameter has been used as a preliminary test to compare NU analogs in regard to their 'operational' therapeutic index [10]. Chlorozotocin (CZT) (or 2–[3-(2-chloroethyl)3-nitrosoureido]-glucopyranose) [11, 12], which we studied in a second experiment, also produced MEDR, whereas BCNU, CCNU and GCNU did not; RPCNU produced a short MEDR, while RFCNU gave the longest range of effective doses [9, 13].

RFCNU produced excellent antitumor activity in several transplanted murine leukemias and solid tumors [9, 13]. It is only moderately toxic to hemopoietic stem cells (CFU-s), and this effect is rapidly reversible. There is only moderated and reversible acute toxicity on granulopoietic precursors (CFU-c) [14], and the hematological toxicity in the mouse is not cumulative [15]. RFCNU is not immunosuppressive, and the dose modality of application, optimally oncostatic on L1210 leukemia, was found able to potentiate delayed hypersensitivity and macrophage cytostatic activity [16]. It is moderately mutagenic [17], which may be the result of its relatively low alkylating activity (40% of chloroethyl nitrosourea CNU) compared to that of CZT (64%) and that of RPCNU (11%) (Schein, personal communication).

RFCNU

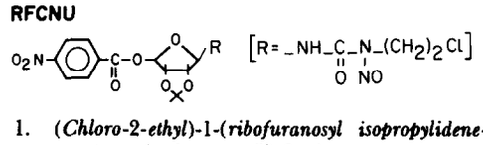

Fig. 1. *(Chloro-2-ethyl)-1-(ribofuranosyl isopropylidene-2'-3 paranitrobenzoate-5')-3 nitrosourea.*

RFCNU (as RPCNU) blocks cells in G_2-M for 24 hr at the respective doses of 20 μg and 50 μg/ml, while CZT (at the dose of 20 μg/ml) blocks them for 72 hr [18].

MATERIALS

Patients

We report here the treatment results for two categories of tumors: DT and carcinomas of unknown origin.

The patients were entered into study according to the general rules for phase II trials [19]. They were in an advanced stage of their disease and had one or more measurable metastases, or a locally recurrent primary localization not amenable to surgery or radiotherapy. Eighteen patients had been previously treated and were resistant to chemotherapy; six had already received an NU analog.

Eligibility criteria were: (a) the histological confirmation of malignancy; (b) the evidence of tumor progression under all previous therapies and/or the absence of any effective therapy; (c) the presence of measurable disease; (d) the possibility of adequate follow-up.

Fifty-seven patients presenting these criteria are available for result evaluation (Table 1). There are 32 men and 25 women with a mean age of 54 years (range 26–74 years).

Antitumor response was classified as a complete response (CR) or partial regression (PR) greater than 50% of the sum of the products of the diameters of measured lesions and lasting more than 6 weeks. There could not be a simultaneous increase in size of any other lesions or appearance of new localizations. Minor responses were also noted, which included regressions between 50 and 30% with objectively measurable lesions [19, 20].

Table 1. *Treatment with RFCNU of digestive tract tumors and of metastatic tumors of unknown origin*

No. evaluable patients	57
Age	54 yr
Mean	26–74 yr
Range	26–74 yr
Sex	
Male	32
Female	25
No. resistant to previous chemotherapy (NU)	18 (6)

Only the patients with platelet counts greater than 15×10^4 mm^3, with more than 25×10^2 polymorphonuclears per mm^3 and more than 11 g hemoglobin per 100 ml were considered suitable for the evaluation of hematological toxicity. Thrombocytopenia was considered as significant when the platelet count was less than 10^5/mm^3, and severe when it was below 5×10^4/mm^3; anemia was considered as significant when the hemoglobin level was less than 10 g/100 ml, and neutropenia when the number of polymorphs was below 15×10^2/mm^3.

As RFCNU is not a water-soluble compound it was given orally. The conduct of the trial was as follows: the selected patients received a monthly dose divided into two days, following a hematological control performed on the first day. A phase I trial [21] has indicated that the optimal dose was between 300 and 350 mg/m^2/month, which was also the dose designated in a baboon preclinical toxicity systematic study [22]. We started the phase II trial at this dose and, when tolerance was excellent, we further escalated it up to 500–600 mg/m^2/month in a few patients with a maximum dose of 750 mg/m^2/month in one case. In patients with previous bone marrow insufficiency we administered doses below 200 mg/m^2/month.

Treatment was delayed in the case of a platelet number below 50,000/mm^3 and/or a granulocyte number less than 1500/mm^3 the day before the cycle. It never had to be interrupted for other side-effects.

RESULTS

As seen on Table 2, we obtained 8 objective responses (14%): 3 complete responses were obtained (one of a pancreatic carcinoma with metastasis in the liver, one on an adenocarcinoma of unknown origin metastasized in the liver, and one with a similar tumor metastatic to the lung), and 5 partial remissions were also recorded (Table 2). In addition, 8 minor responses were registered. The disease progressed under treatment in 41 patients.

Table 3 shows the characteristics of the responding patients: 4 patients with liver metastases (with 2 CR), 3 patients with lung metastasis (1 CR) and one rectal tumor. The median duration of the response was 9 months, with a range of 2–44+ months. The lowest effective dose was 275 mg/m^2 per cycle. There is no clear correlation between the dose administered and the antitumor effect (Table 4).

Toxicity

Eight patients (14%) presented nausea and/or vomiting (Table 5).

The hematologic toxicity reported in Tables 5, 6 and 7 was registered on 50 patients and was defined as in Materials and Methods: anemia (2%), neutropenia < 1500/mm^3 (4%), 9 thrombocytopenias < 100,000/mm^3 (18%) and 3 < 50,000/mm^3 (6%). This toxicity was not directly related to the number of cycles administered but it was directly correlated with prior treatment. No severe hematologic toxicity was observed among the 32 previously untreated patients. Severe thrombocytopenia occurred only in those patients previously treated with chemotherapy. One patient died from thrombocytopenia who had been previously treated with RPCNU.

Table 2. Therapeutic activity of RFCNU on digestive tract (DT) tumors and tumors of unknown origin

Diagnosis of primary tumor		No. of patients	Major responses* CR	Major responses* PR	Minor responses†	Progressive disease
Colorectal	2	32	—	1	1	—
	Liver metastases 7		—	—	1	6
	Lung metastases 18		—	—	2	16
	5		—	2	—	3
Pancreas		6	1	—	—	5
Gall bladder		1	—	—	—	1
Liver		1	—	—	1	—
Esophagus		1	—	—	—	1
Stomach		3	—	—	1	2
Unknown	Adenocarcinomas	10	2	1	1	6
	Undifferentiated carcinomas	3	—	1	1	1
Total		57	3	5	8	41

*>50% according to WHO/EORTC/NCI report [19].
†<50%.

Table 3. Characteristics of responders to RFCNU

No.	Age/sex	Tumor type	Localization of responses	Type of response	Dose mg/m²/cycle	Duration of response
1	60/M	Undifferentiated carcinoma; unknown primary	Liver	PR	275	8 months
2	72/F	Rectum	Lung	PR	300	2 months
3	74/M	Adenocarcinoma; unknown	Liver	PR	375	15 months
4	45/F	Adenocarcinoma; unknown primary	Lung	CR	375	18 months
5	59/F	Rectum	Tumor	PR	470	9 months
6	60/F	Rectum	Lung	PR	512	9 months
7	38/F	Adenocarcinoma; unknown primary	Liver	CR	533	7 months
8	55/M	Pancreas	Liver and tumor	CR	570	44 + months

Table 4. RFCNU response as a function of initial dose

Initial dose (mg/m²)	No. evaluable	Responses
150–199	3	0
200–299	16	1
300–399	15	3
400–499	16	1
500–599	6	3
750	1	0

Table 5. RFCNU toxicity

No. of evaluable patients	Hematologic				Digestive nausea/vomiting
	Thrombopenia 100,000/m³	Thrombopenia 50,000/m³	Neutropenia <1,500/m³	Anemia <10 g/l	
50	9	3*	2	1	7
	18%	6%	4%	2%	14%

*Lethal: prior treatment with RPCNU 1 month before.

Table 6. RFCNU hematologic toxicity according to number of cycle administered

No. of cycle	No. of evaluable patients	Thrombopenia	Leucopenia	Anemia
1st Cycle	50	3	1	0
2nd Cycle	47	4	1	1
3rd Cycle	28	2	0	0

Dose: 150–750 mg/m²/month. $n = 50$.

DISCUSSION

The remission rate observed with RFCNU in this trial (14%) is comparable to that reported for BCNU (13%) [1], CCNU (7%) [2] and MeCCNU (11%) [3]. Liver and lung metastases seem to be sensitive targets: 4 major regressions in 15 patients with liver metastases and 3 major regressions in 6 patients with lung metastases. The length of some remissions should be noted: the median duration was 9 months, with one CR lasting more than 44 months.

Table 7. RFCNU hematologic toxicity according to prior treatment

Prior chemotherapy	No. of evaluable patients	Thrombopenia	Leucopenia	Anemia
None	32	4 (12%)	1	0
Nitrosourea	6	2* (33%)	1	1
Others	12	3 (25%)	0	0

*Lethal: prior treatment with RPCNU 1 month before.

The toxicity of RFCNU was moderate, which made its easier to use in our experience than that of any classical analog [1–4]. Moreover, we observed only 14% digestive side-effects (nausea and vomiting), compared to 55% reported for CCNU [2] and 58% for MeCCNU [3]. We have only registered anemia in 2% of the patients, and severe neutropenia in 4%. RFCNU appears to be less toxic for neutrophils than BCNU, CCNU and MeCCNU [1–3], but this can only be objectively examined in a controlled comparative trial. Finally, when we compared the respective incidences of thrombocytopenia of $< 100{,}000/mm^3$, this was found in 18% of patients with RFCNU vs 66–68% for BCNU, 59% for CCNU and 47–62% for MeCCNU [1–3]. Platelet counts of below $50{,}000/mm^3$ were found in 6% of patients treated with RFCNU, compared to 41% with BCNU, 28% with CCNU and 28% with MeCCNU [1–3]. One patient has remained in CR for 3.5 yr on RFCNU. A dose of 750 mg/m^2 did not result in severe cumulative toxicity; platelet counts have remained over $100{,}000/mm^3$.

The hematologic toxicity of RFCNU is not correlated with the number of cycles administered and cumulative bone-marrow toxicity is not evident, as is the case for first generation NU. It should also be remembered here that 18 RFCNU patients had previously been submitted to chemotherapies, among which 6 had received NU analog(s).

Thus RFCNU appears to be less hematotoxic and especially less thrombocytotoxic than BCNU, CCNU and MeCCNU.

In conclusion, this phase II trial demonstrates that RFCNU is an active drug in colorectal tumors and metastatic carcinomas of unknown origin, with response rates similar to that of classical NU analogs [1–4]. In addition, a number of minor regressions have been documented. The dose-limiting toxicity appears to be bone-marrow-delayed depression. This toxicity is not strictly dose-dependent. One possible explanation may be incomplete and variable absorption of RFCNU from the digestive tract, but this possibility will require future pharmacokinetic analysis. Furthermore, the drug is probably rapidly hydrolysed at the acidic pH of gastric juice, liberating the unsubstituted ribosylchloroethyl nitrosourea [23].

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