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Phase I Clinical Study of the New Amino Acid-linked Nitrosourea, S 10036, Administered on a Weekly Schedule

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ABSTRACT

Diethyl-1-[3-(2-chloroethyl)-3-nitrosoureido]ethylphosphonate (S 10036) is a new nitrosourea that has been evaluated in a clinical trial because of its activity in the National Cancer Institute panel screen and its rational chemical approach.

A Phase I study was conducted in 22 evaluable patients with advanced cancers. The drug was given as a slow i.v. infusion over a period of 60 min on days 1, 8, 15, and 22 followed by a 4-week rest period. The dose levels ranged from 25 to 200 mg/m²/week for 4 consecutive weeks using a modified Fibonacci scheme. Thrombocytopenia was the only acute dose-limiting toxicity and started at a dose of 100 mg/m²/week and above. Hematological toxicity was delayed, cumulative, and dose related. Nausea and vomiting were moderate to severe and dose related. Three responses (one complete and two partials) have been noted.

Phase II studies of S 10036 are planned at a dose of 100 mg/m²/week for 4 consecutive weeks ("induction therapy") for patients without prior therapy and 100 mg/m²/week for 3 consecutive weeks for those with prior chemotherapy or radiotherapy.

Because of the cumulative toxicity, the recommended dose for the second cycle of S 10036 chemotherapy ("maintenance therapy") is 100 mg/m²/week every 3 weeks.

INTRODUCTION

Chloroethylnitrosoureas are a group of anticancer agents with a high demonstrated activity against a variety of hematological malignancies and solid tumors, especially Hodgkin's disease, small cell carcinoma of the lung and glioma, but with a clinical use rather limited due to their delayed and cumulative hematological toxicity (1). The chemistry of these compounds have been extensively studied (2, 3) and a variety of structural modifications have been tested, aimed at optimizing their activity against murine leukemia and/or at decreasing their hematological toxicity.

Recently, Servier Research Institute has synthesized several amino acid congeners based on quantitative structure-activity relationships (4, 5). Among them, the nitrosourea S 10036² (Fig. 1), which contains a phosphonoalanine carrier group grafted to the nitrosourea radical, has been synthesized and its activity carefully studied according to the guidelines of the National Cancer Institute antitumor screen: S 10036 was active in P 388, L1210 leukemia, colon 26, Lewis lung carcinoma, Sarcoma M 5076 and B 16 melanoma.

As expected for a nitrosourea compound, the toxicological studies after i.p. administration in mice and Beagle dogs showed that S 10036 was mainly toxic to the hematopoietic system; the single-dose LD₅₀ values (dose which kills 50% of the animals tested) in male and female Swiss mice were 60.1 and 61.3 mg/kg, respectively. Several of its characteristics indicated that

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² The trivial name used is: S 10036, diethyl-1-[3-(2-chloroethyl)-3-nitrosoureido]ethylphosphonate.

amino acid-linked nitrosourea S 10036 was a good candidate for further clinical studies: its high experimental activity on the National Cancer Institute screening system, the rational of its chemical synthesis approach with the grafting of an amino acid carrier contributing to the cellular penetration of the drug, a log *P* value of 1.25 which is within the limits of the maximum antitumor activity (-0.20 to +1.34) as evaluated by the structure-activity relationship studies, and its lower mutagenicity in both Ames and micronucleus tests when compared to 1,3-bis(2-chloroethyl)-1-nitrosourea. Therefore, a Phase I clinical study has been initiated aimed at identifying (a) the characteristics of the toxicity of the nitrosourea S 10036 administered on a weekly schedule and (b) the optimal dose for further clinical trials.

MATERIALS AND METHODS

Patient Selection. All patients who entered the trial had a histologically confirmed cancer which had proven to be resistant to existing conventional therapeutic treatments. Informed consent was obtained from all patients. To be eligible, patients were between 18 and 75 years old, presenting any kind of malignant tumor. They must have discontinued therapy prior to the administration of S 10036 for at least 28 days (prior nitrosourea treatment was a criteria of exclusion). They had to have a life expectancy of at least 8 weeks, a performance status greater than 50% on the Karnofsky scale, a WBC greater than 4,000 cells/mm³, a platelet count greater than 100,000 cells/mm³, a normal liver function (based on transaminases and bilirubin) unless abnormalities were clearly due to metastatic disease, and a normal renal function (blood urea and creatinine). During treatment the patients had a weekly clinical examination including WBC with platelet counts, hemoglobin and hematocrit, transaminases, bilirubin, serum creatinine, and blood urea. Electrocardiograms as well as other laboratory, radiological, and isotopic studies used for tumor evaluation were performed before treatment and at weeks 4 and 8.

Treatment Plan. S 10036, formulated in sterile vials of 100 mg/5 ml ethanol (95% v/v), proved to be stable for at least 6 months under refrigeration as determined by high-performance liquid chromatographic analysis. The vials were diluted in 5% glucose (250 ml) and the solution was protected from light before and during administration: under these conditions the solution was stable for at least 4 h.³ The solution was infused i.v. over a 60-min period, this treatment being repeated every week for 4 consecutive weeks unless severe toxicity was observed. The starting dose was 25 mg/m²/week (1/6 of mouse equivalent LD₁₀) for 4 consecutive weeks; doses were escalated according to a modified Fibonacci scheme up to 200 mg/m²/week for 4 consecutive weeks. As S 10036 was an analogue, only one patient was entered at the initial dose level, but thereafter doses were escalated only when at least two or three patients had completed their treatment plan at the previous level.

Toxicity. Clinical, hematological, hepatic, and renal toxicities were scored according to WHO classification, and were collected every week for all patients. Electrocardiogram modifications, as well as chest X-ray were recorded every 4 weeks.

Termination of Study. Individual patients were taken off the study if an objective tumor progression occurred following one cycle of 4 weekly

³ F. Lokiec and J. P. Bizzari, unpublished data.

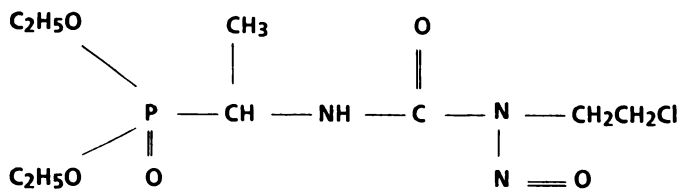


Fig. 1. Structural formula of diethyl-1-[3-(2-chloroethyl)-3-nitrosoimido]ethylphosphonate.

Table 1 Patients' characteristics

Characteristics	Number of patients
Evaluable patients	22
Males/females	15/7
Age (years)	
Median, 53.4	
Range, 25-65	
Treatment history	
No prior treatment	1
Chemotherapy	21
Prior nitrosourea	0
Radiotherapy	8
Radio and chemotherapy	8
Tumor type	
Chronic lymphocytic leukemia	1
Colorectal	3
Breast	2
Ovary	1
Melanoma	7
Myeloma	1
Sarcoma	1
Lung	4
Adenocarcinoma (2)	
Anaplastic (1)	
Squamous (1)	
Thyroid	1
Unknown origin	1

injections of S 10036. Due to the expected cumulative toxicity of S 10036 (as it is a nitrosourea analogue), only one cycle of such weekly administration was planned in each patient. When a response was obtained (minor, partial, or complete), the treatment plan was continued with a single infusion every 3 weeks starting 4 weeks after the last weekly infusion of the first four-dose cycle. However, cumulative toxicity of weekly infusions was observed in one patient whom responded to S 10036 therapy and who was treated for a second 4-week cycle.

RESULTS

Over a 12-month period, 26 patients were entered into the S 10036 Phase I trial. Among them, 22 patients were evaluable for toxicity and 16 were evaluable for response. Four patients (15.4%) were nonevaluable; two died before the end of the first 4 weeks of treatment due to rapid tumor progression and two were lost to follow-up after 2 and 4 weeks, respectively. Patient characteristics are summarized in Table 1. Among the 22 evaluable patients, 18 received the four weekly scheduled injections as indicated on the treatment plan, three patients received only three weekly injections [one patient experienced an early thrombocytopenia, one patient decided to discontinue chemotherapy, and one patient (at the highest dose, 200 mg/m²/week) discontinued for ethical reasons since the cumulative dose after three injections was equivalent to the 600 mg/m² maximum-tolerated cumulative dose that had already been determined from four weekly injections of 150 mg/m²], one patient received only two injections because of borderline thrombocytopenia at the beginning of treatment.

A total of 83 courses of S 10036 were given and were

evaluable for toxicity. A modified Fibonacci scheme was used; courses per dose level and per patient are listed in Table 2. Up to the third dose level of 75 mg/m²/week, no major side effect or dose-limiting toxicity was seen.

Hematological Toxicity. Myelosuppression, particularly thrombocytopenia, was the major side effect and the dose-limiting toxicity of S 10036. This occurred following weekly administration of doses of 100 mg/m² (total cumulative dose, 400 mg/m²) and above. The characteristics of this myelosuppression are clearly indicated in Table 3; the three elements (RBC, WBC, and platelet counts) were involved. This myelosuppression was dose related, starting at around 75 mg/m²/week (cumulated dose, 300 mg/m²). At 150 mg/m² thrombocytopenia occurred in both patients. The myelosuppression was delayed with thrombocytopenia starting at day 22 with a nadir at day 35 and recovery by day 47; leukopenia started at day 36, the nadir being at day 42 and recovery by day 47; anemia also started at day 24, with a nadir around day 35. Increasing dose does not appear to influence the time of onset and recovery of the myelosuppression. Moreover, this hematological toxicity seems to be cumulative as observed in one patient (number 17) who was treated with a second cycle of four weekly doses of S 10036 after a 4-week interval [nadir of thrombocytopenia at cycle one, 70,000/mm³ versus 25,000/mm³ at cycle two; nadir of hemoglobin, 9.6 g/100 ml at cycle one versus 6.6 g/100 ml at cycle two; no change for WBC count (1,400/mm³)].

Gastrointestinal Toxicity. As shown in Table 4, nausea and vomiting, starting 2-6 h after the end of the infusion and lasting for 2-4 h, were observed with a dose-effect relationship and were dose limiting. Up to a weekly dose of 125 mg/m², no antiemetics were required prophylactically, but then nausea and vomiting became severe (125 mg/m², one patient out of three with grade IV toxicity) and metoclopramide (40-60 mg/day) was given prophylactically to all patients. Even so, severe gastrointestinal toxicity was observed at higher doses (150 and 200 mg/m²).

Other Side Effects. No other side effects, except rare and transient elevated γ -glutamyl transferase and/or transaminases at doses higher than 82.5 mg/m²/week were noted. No pulmonary or renal toxicity was observed. Serial electrocardiograms did not show any perturbation.

Tumor Response. One complete response and two partial responses according to the WHO classification were observed during this Phase I study among the 16 patients evaluable for response. The complete response was observed in patient 1 with chronic lymphocytic leukemia and who had previously progressed under treatment with cyclophosphamide, hydroxydaunorubicin, vincristine, prednisone (CHOP) and CHOP bleomycin regimens. The two partial responses were observed in melanoma patients: one patient had a volume decrease of >50% of his node metastasis, the other patient experienced >50% volume decrease of his nodes and cutaneous metastasis. Two minor responses have also been evaluated, one on a breast cancer-related pleural effusion and the second on malignant melanoma brain metastasis.

DISCUSSION

Numerous analogues of nitrosoureas have been synthesized based on a structure-activity relationship aimed at increasing their antitumor activity and/or decreasing their hematopoietic toxicity.

The concept for creating the S 10036 compound was based on pharmacodynamic considerations. Indeed, the grafting of an

Table 2 Dose level and patients' characteristics of the Phase I study of S 10036

S 10036 Dose (mg/m ² /week)	Courses (number of weeks)	Patients (number)	Sex	Age (years)	Tumor	Site of metastasis	Prior treatment ^a		
							SG	RT	CT
25	4	1	M	52	CLL ^b	Nodes	No	No	Yes
50	4	2	F	63	Sigmoide	Lung and liver	Yes	Yes	Yes
	4	3	F	42	Breast	Lung and liver	No	Yes	Yes
75	4	4	M	47	Sarcoma	Local	Yes	No	Yes
	4	5	M	62	Myeloma	Bone	No	No	Yes
82.5	4	6	M	52	Unknown	Bone	No	No	Yes
	4	7	F	54	Breast		No	No	Yes
	4	8	M	42	Lung (adenocarcinoma)	Nodes	Yes	Yes	Yes
100	4	9	M	53	Colon	Lung	Yes	No	Yes
	4	10	M	65	Lung (small cell)	Brain	No	Yes	Yes
	4	11	M	64	Thyroid	Lung	Yes	Yes	Yes
	4	12	M	61	Lung	Local and bone	Yes	Yes	Yes
	3	13	M	65	Lung (adenocarcinoma)	Lung	No	Yes	Yes
	4	14	M	63	Lung (adenocarcinoma)	Lung	Yes	No	Yes
	2	15	F	42	Colon	Liver and nodes	Yes	No	Yes
	3	16	M	54	Melanoma		Yes	No	Yes
125	4	17	M	51	Melanoma	Skin	Yes	No	Yes
	4	18	M	25	Melanoma	Nodes	No	No	No
	4	19	F	60	Ovary	Lung	Yes	No	Yes
150	4	20	F	59	Melanoma	Liver	Yes	No	Yes
	4	21	F	52	Melanoma	Nodes and liver	No	No	Yes
200	3	22	M	47	Melanoma	Brain and nodes	Yes	Yes	Yes

^a SG, surgery; RT, radiotherapy; CT, chemotherapy.
^b CLL, chronic lymphocytic leukemia.

Table 3 Hematological toxicity of S 10036

Dose (mg/m ² /week)	25	50	75	82.5	100	125	150	200	Start	Nadir	Recovery	
Number of patients	1	2	2	3	8	3	2	1				
	<i>Leukopenia (/mm³)</i>											
≥4000	●	●	●	●●●	●●●●				Day 36 (32-43) ^b	Day 42 (38-47)	Day 47 (44-54)	
3000-4000					●			●				
2000-3000		●			●●●	●●●	●					
2000-1000			●				●					
<1000												
	<i>Thrombocytopenia (/mm³)</i>											
≥100,000		●●	●	●	●				Day 22 (17-25)	Day 35 (31-39)	Day 47 (43-56)	
75,000-100,000	●		●	●	●	●						
50,000-75,000					●	●						
25,000-50,000				●	●●●	●						
<25,000					●●	●	●●	●				
	<i>Anemia (hemoglobin, g/100 ml)</i>											
≥11 g/100 ml	●	●		●●	●●●	●						
9.5-11		●		●	●●●	●	●					
8-9.5			●	●	●	●	●	●				
6.5-8					●							
<6.5												

●, one patient.
^b Numbers in parentheses, range.

Table 4 Gastrointestinal toxicity of S 10036

Dose (mg/m ² /week)	25	50	75	82.5	100	125	150	200	Start
Number of patients	1	2	2	3	8	3	2	1	
Nausea/vomiting									
None	●●●●●●●●	●●							2-6 h After perfusion
Mild		●●	●●●●●●●●						
Moderate					●				
Severe						●●●●			

●, one patient.

aminophosphonic acid (moreover, in the form of diethyl ester in order to increase solubility) onto the nitrosourea radical was theoretically planned to achieve both a better penetration through the cell membrane and a better antitumor activity; as a matter of fact, differences in membrane permeabilities to amino acids have been found between healthy and some cancer cells (6) and the transport of drugs into cells by neutral amino

acid transport systems (7, 8) has been demonstrated for the L-phenylalanine mustard in which the nitrogen mustard moiety is attached to an L-phenylalanine. Whether such a cell membrane transport mechanism for S 10036 exists *in vivo* is still unclear, studies on tumor-bearing animals and on cell cultures with radiolabeled S 10036 molecule are in progress.

We report here the results of the Phase I clinical study. Myelosuppression was the major side effect and thrombocytopenia the only acute dose-limiting toxicity. As expected, the type of hematological toxicity of S 10036 seems to be similar to that of other nitrosoureas since the myelosuppression was dose related, delayed, and cumulative (9).

The weekly regimen used in the present study was chosen in order to evaluate a possible schedule-dependent pattern of toxicity, as observed in animal studies.⁴ In addition, it was

⁴ Unpublished results.

expected that the fractionation of the total dose would prevent partially the myelosuppression and/or increase the response rate by giving higher total doses of the cytotoxic drug within the 8-week time interval ("induction therapy"). Although this regimen did not show any toxicological advantage, it may explain the unusual and unexpected high response rate observed in this Phase I trial. The nonhematological toxicity of S 10036 was limited in this study to nausea and vomiting which were dose dependent.

Based on these results, especially on the responses observed in metastatic malignant melanoma, Phase II clinical trials are now planned in a variety of solid tumors and particularly in malignant melanoma. The recommended starting dose for the first cycle ("induction therapy") is 100 mg/m²/week for 4 consecutive weeks (cumulated dose, 400 mg/m²) in previously untreated, good performance status patients while patients who present a poor performance status and/or have had a prior bone marrow toxic chemotherapy or radiotherapy should be treated at a dose of 100 mg/m²/week for 3 consecutive weeks (cumulated dose, 300 mg/m²).

If this "induction therapy" is successful (clinical response or at least stabilization in otherwise progressive disease), then a

maintenance therapy at a dose of 100 mg/m² every 3 weeks seems to be legitimate.

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