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Phase II Evaluation of Chlorozotocin (NSC-178248) in Advanced Human Cancer*†

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Abstract—*A phase* II *evaluation of chlorozoticin (CZT), a water soluble nitrosourea, was undertaken to determine its effectiveness and toxicity in a variety of human metastatic neoplasms. The dosage regimen chosen was either* 90 *or* 120 mg/m^2 *given by i.v. bolus every six weeks. Dosage escalation or de-escalation was dependent on toxicity. There have been* 152 *patients evaluable for response. The only significant response rates observed were in non-Hodgkin's lymphoma* (5/11) *and sarcoma* (4/27). *Single responses were observed in breast and oat cell carcinoma of lung. No responses were observed in melanoma, colorectal, kidney, non-oat cell lung, pancreas, stomach and other carcinomas. Hematological toxicity has been minimal as predicted, but does appear to be cumulative. The major G.I. toxicity has been nausea and vomiting—usually controllable. Occasional hepatic enzyme elevations were observed, and azotemia was observed in* 6 *patients. Both were reversible. Rare skin and occasional CNS reactions were also seen.*

INTRODUCTION

CHLOROZOTOCIN (CZT) is a nitrosourea recently synthesized by Johnson *et al.* [1] and related chemically to streptozotocin (STZ) and the chloroethyl nitrosourea BCNU. Both STZ and CZT are attached to a glucose carrier at the carbon 2 position. Whereas STZ is a methyl nitrosourea, CZT is a chloroethyl nitrosourea, as are CCNU, BCNU and methyl CCNU. The latter three compounds have had extensive clinical trials and have shown activity against a wide variety of human cancers such as melanomas, lymphomas, lung, breast and gastrointestinal carcinomas and gliomas. Their usefulness has been limited by progressive myelosuppressive toxicity. STZ has had very little myelosuppression associated with its use, but its clinical usefulness has been limited to islet cell tumors of the pancreas. CZT and STZ are both water soluble, but BCNU, CCNU and methyl CCNU are lipid soluble. The latter compounds have strong alkylating and carbamoylating activities, whereas both STZ and CZT have high alkylating and low carbamoylating activities [2].

Studies in the transplanted L-1210 leukemia revealed CZT to have equal antitumour effect to BCNU but significantly less myelosuppression as demonstrated by decrease in peripheral white blood cells [3]. However, at equitoxic doses BCNU-treated mice with L-1210 leukemia had an increased number of 90-day survivors. The same lack of myelosuppression was also shown by *in vitro* studies of human bone marrow cells exposed to CZT as compared to BCNU [2].

After appropriate animal toxicity studies, CZT was then applied to humans with advanced cancer in Phase I clinical trials. Hoth *et al.* [4] found that CZT administered to patients in a dose-escalating regime of 5–175 mg/m^2 produced no myelosuppression at doses below 120 mg/m^2. Their treatment schedule was to administer CZT as an i.v. bolus injection every 6 weeks. Even at doses of 120–150 mg/m^2 leukopenia was minimal and thrombocytopenia achieved moderate levels of depression in patients receiving 2–4 courses. Hoth *et al.* observed nausea in 40% of their patients. Transient elevation of SGOT and

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SGPT were also noted in about 1/4 of patients, but no renal toxicity was observed. Hyperglycemia was not observed. Three patients with metastatic melanoma were observed to have partial objective responses, as was one patient with adenocarcinoma, all of which were of short duration.

Gralla *et al.* [5] administered CZT in a dose-escalating schedule of 75–200 mg/m^2 divided into 5 equal daily doses every 6 weeks. They also observed that myelosuppression was their dose-limiting toxicity at doses of 150 mg/m^2 or greater. Thrombocytopenia was relatively greater than leukopenia. No hepatic or renal toxicity or hyperglycemia was observed. One patient developed a petechial rash without thrombocytopenia. Only 6 minor regressions were observed in the 31 patients.

Because of these studies suggesting that myelosuppression was less, we undertook an evaluation of CZT in a large number of patients with a wide variety of advanced malignancies, utilizing the single i.v. injection every 6 weeks.

METHODS

This protocol was opened for patient entry in November 1977, and this report covers all patients entered through November 1979. Patients were entered on the study if:

(1) all effective methods of treatment had been utilized or no effective therapy was available for their disease;

(2) measurable disease was present;

(3) predicted survival was 8 weeks or greater;

(4) they had recovered from prior chemotherapy;

(5) WBC was $>4000/mm^3$ and platelets were $>100{,}000/mm^3$.

An informed consent signed by the patient or the legal guardian was required for all patients prior to entry on the study. The signal neoplasms to be studied were carcinoma of the breast, colon, and lymphoma, melanoma and sarcoma. However, other malignancies could be entered. All patients were to receive pre-treatment and weekly CBC and platelet counts. Biochemical evaluation of renal, hepatic and pancreatic islet function as well as clinical evaluation with appropriate measurement were performed at 3-week intervals.

CZT was supplied in 50 mg vials and was reconstituted with 5 ml of sterile water or normal sodium chloride just prior to administration by a bolus i.v. injection.

Initially, patients were divided into good risk and poor risk groups, but in October 1978 the poor risk (greater than 65 years of age) category was eliminated and all patients were subsequently entered at the good risk dose level if all requirements were met. Good risk patients received 120 mg/m^2 and poor risk patients received 90 mg/m^2. Patients were to be re-treated at 6-week intervals. Subsequent dosage was decreased by 30 mg/m^2 if thrombocytopenia of $<75{,}000$ and/or leukopenia of <2500 was observed. Subsequent dosage was to be increased by 30 mg/m^2 if WBC remained above 4000 and platelets above 100,000 and no clinical response was observed.

Responses were recorded as follows:

(1) complete—disappearance of all clinical evidence of disease for 4 weeks or more;

(2) partial—50% or greater decrease in the sum of the products of the perpendicular diameters of measurable lesions;

(3) no change—50% decrease in measurements but >25% increase and no new lesions;

(4) increasing disease—increase 25% of measurements or the appearance of any measurable lesion.

Patients were considered evaluable if they received one dose of the drug and were followed for a minimum of 6 weeks.

RESULTS

Accrual and responses

Patient accrual during the period of this study (Table 1) was a total of 184 patients, 11 of whom are ineligible, leaving 173 eligible patients. One hundred and twenty five of these patients were considered good risk and 48 were poor risk, but as there were no significant differences in toxicities or responses in these groups, the data presented subsequently will not separate these 2 groups.

Table 1. *Chlorozotocin—phase* II *study*
Patient accrual data

No. entered	184
Eligible	173
Ineligible	11
Fully evaluable	144
Not evaluable	13
Partially evaluable	16
Response only	8
Toxicity only	8

At the time of this report 144 patients are fully evaluable and 16 are partially evaluable, 8 for toxicity only or 8 for response only. Of the 13 non-evaluable patients 2 are too soon, 6 had early death, and 5 were non-evaluable for other reasons. Twenty-seven patients are still on study.

Table 2. Chlorozotocin—phase II study. Patient characteristics

Age range	29—79 yr	(Median 59 yr)
Sex		
Male 81, Female 103		
Performance status		
≧40	17	
45–60	46	
65–100	101	
Unknown	20	
Prior treatment		
Chemotherapy only	87	
Radiation therapy only	9	
Radiation therapy and chemotherapy	75	
No radiation or chemotherapy	12	
Unknown	1	

Patient characteristics of all eligible patients are shown in Table 2. The majority of patients had a favorable performance of 65 Karnofsky scale or better, attesting to the low number of early deaths mentioned above. Extensive chemotherapy, radiotherapy or both had been carried out prior to entry on the study. This probably influenced the low response rates observed and perhaps the toxicity observations also.

A wide variety of patients were entered, as shown in Table 3. The percentage of responders shown in Table 3 applies to the evaluable patients only, not the eligible patients entered. All responses were partial. The number of evaluable patients in the categories in which partial responses were observed were: (1) breast, 25; (2) lung, 25; (3) lymphoma, 11; and (4) sarcoma, 24. Colorectal carcinoma was studied extensively because of lack of an effective agent other than fluorouracil in this tumor. Sixteen of the 45 patients entered with colorectal cancer had had no prior chemotherapy.

Thus the criteria of entering at least 14 patients in the signal tumor categories in order to determine the effectiveness of the drug has been met in all the signal tumor categories except for melanoma. The most interesting responses have been observed in the lymphomas and sarcomas, all of whom had had prior extensive chemotherapy and/or radiotherapy.

The more definitive diagnoses of the responding patients is given in Table 4. All of the lymphoma responders were of the diffuse non-Hodgkin's type. No evaluable Hodgkin's disease patients were included in this group. Responses in the sarcoma group represent 3 soft tissue sarcomas and one sarcoma of bone origin. One of four oat cell carcinomas of the lung responded. This patient died from a myocardial infarction 3.4 weeks after his second dose of the drug at 120 mg/m^2. This was not drug-related.

Table 3. Chlorozotocin—phase II study. Patient entry by primary site and response

Primary site	Number entered	Number eligible	Number evaluable for response	Responders % eval./ No. resp.
Breast	28	28	25	1 (3.6%)
Colorectal	45	41	34	0
Kidney	9	9	8	0
Lung	28	27	25	1 (3.6%)
Lymphoma, non-Hodgkin's	15	13	11	5 (45%)
Melanoma	10	9	8	0
Pancreas	7	6	5	0
Sarcomas	27	27	25	4 (16%)
Head and neck	5	5	4	0
Stomach	4	3	3	0
Miscellaneous	6	5	4	0
	184	173	152	11

Table 4. *Chlorozotocin—phase* II *study. Responders*

Patient	Age	Sex	Diagnosis	Total courses	Duration of response (weeks)
E.C.	71	M	Diff. lympho-cytic lymphoma	22	141
A.B.	61	M	Diff. undiff. lymphoma	8	48
J.C.	62	M	Diff. undiff. lymphoma	3	9
E.J.	68	M	Diff. undiff. lymphoma	2	4
J.P.	71	M	Diff. undiff. lymphoma	3	9
M.B.	58	F	Breast adenoca.	5	20
M.V.	57	F	Leiomyosarcoma	8	32
L.Y.	67	M	Fibrosarcoma	12	60+
S.G.	29	F	Giant cell sarcoma	12	50+
C.M.	26	F	Leiomyosarcoma	4	14
H.C.	60	M	Oat cell lung	2	3

Toxicity

The incidence of leukopenia and thrombopenia occurring in these patients are presented in Table 5. Definition of these toxicities are:

None—WBC > 4000; platelets > 100,000

Mild—WBC 3000–3999; platelets 75,000–99,999

Moderate—WBC 2000–2999; platelets 50,000–74,999

Severe—WBC 1000–1999; platelets 25,000 49,999

Life threatening—WBC < 1000; platelets < 25,000.

Table 5. *Chlorozotocin—phase* II *study. Hematologic toxicity*

	Leukopenia	Thrombopenia
None	98	100
Mild	33	15
Moderate	12	14
Severe	7	13
Life-threatening	0	3

As can be seen, the incidence of severe or life-threatening leukopenia was uncommon whereas there were more incidences of severe or life-threatening thrombopenia. However, these were not associated with any hemorrhagic phenomenon. There appeared to be an increase in hematopoietic toxicity in these patients with increasing exposure to subsequent courses of the drug; this will be discussed later.

Only 3 patients experienced a < 2.0 mg% drop in hemoglobin and it could not be ascertained if this was drug or disease related.

Table 6. *Chlorozotocin—phase* II *study. Gastrointestinal toxicity*

	Vomiting	Diarrhea	Stomatitis	Anorexia
None	92	142	144	144
Mild	34	4	4	8
Moderate	18	2	0	6
Severe	4	0	0	0

Gastrointestinal toxicity (Table 6) occurred with greater frequency than in the previous reports. Nausea and vomiting occurred in varying degrees of severity in approximately 1/3 of the patients, and in 4 patients was severe enough to require discontinuance of the therapy. Diarrhea and stomatitis, though uncommon, did occur and was felt to be drug-related. Anorexia, though not common, has been prolonged in several patients on long-term therapy and in two instances has been associated with moderate or severe liver enzyme elevations.

Table 7 presents incidence of observed hepatic, renal, CNS and skin toxicities. The hepatic abnormalities observed are only elevation of SGOT and/or SGPT, and except for the two patients mentioned above, have not been associated with any symptoms. However, in all instances the enzymes have returned to normal levels at 6–7 weeks post-therapy. Renal toxicity has been only elevation of BUN to levels of 45 mg% or less, or creatinine

Table 7. Chlorozotocin—phase II study. Miscellaneous toxicity

	Hepatic	Renal	CNS	Skin
None	144	144	134	145
Mild	5	5	13	2
Moderate	1	2	2	1
Severe	1	1	1	0

to levels of less than 3.5 mg%, except in one patient with levels greater than these. In all patients the azotemia was reversible on discontinuance of the drug. No changes were noted on urine examinations except for 2+ proteinuria in the patient with the most severe azotemia. CNS toxicity consisted primarily of confusion episodes lasting 1–5 days post injection of CZT. Skin reactions were erythematous generalized rashes. Petechial rashes were not observed. No incidences were observed in any of the 172 evaluable patients.

As the other chloroethyl nitrosoureas demonstrate progressive hematologic toxicity with increasing doses it was felt that the effect of CZT on peripheral blood elements should be evaluated in patients receiving serial courses of CZT. Table 8 presents this data on the lowest WBC and platelet determinations following each course of the drug. As there was a requirement for dose escalation if no toxicity occurred (defined in Methods) several patients received doses of 150–180 mg/m^2. As most of the patients receiving more than three courses were in the responding groups, there was only limited opportunity to observe many patients on prolonged therapy. Even though leukopenia appeared to be less progressive than

Table 8. Hematologic toxicity by dose and course

Dose level		No. of patients	WBC Median	WBC Range	No. of patients	Platelets Median	Platelets Range
90 mg/m^2							
course	1	39	5	2.5–16.5	36	133	22–540
	2	12	3.9	1.4–7.7	12	126.5	38–205
	3	3	4	3.3–4.8	3	120	74–124
	4	2	4.3	3.8–4.9	2	105.5	61–150
	5	3	5.5	3.1–6.3	3	162	132.5–275
	6	3	4.0	2.0–6.1	3	126.7	90–130
	7	2	3.5	3.0–4.0	1		
	8	2	2.8	2.5–3.0	2	75	50–100
	9	2	3.0	2.5–3.5	2	70	50–90
	10	2	2.5	2.0–3.0	2	60	40–80
	11	1		2.4	1		46
120 mg/m^2							
course	1	97	5.7	1.8–23.8	95	193	28.5–720
	2	28	4.4	1.8–17.5	26	184	69–487.9
	3	10	4.6	3.0–13.7	9	140	79–320
	4	3	5.2	3.9–6.8	3	192	63–215
	5	3	3.5	2.5–4.0	3	90	80–110
	6	3	3.2	3.0–3.5	3	90	80–112
	7	2	3.4		2	89	80–98
	8	1			1		100
	9	1		3.0	1		122
	10	1		2.5	1		88
	11	1		2.8	1		74
150 mg/m^2							
course	1	None			None		
	2	27	5.0	2.9–11.2	27	179	36–560
	3	8	4.8	3.1–7.1	8	135.5	49–250
	4	3	2.9	2.3–4.4	3	86	84–148
	5	2	2.5	2.0–3.8	2	74	58–110
180 mg/m^2							
course	1	None			None		
	2	None			None		
	3	6	4.3	3.8–8.3	6	92.5	30–520
	4	4	4.2	3.5–5.0	4	74	59–100
	5	2	4.8	3.1–6.5	2	84	80–88

thrombocytopenia, both increased with greater exposure of patients to CZT. This is demonstrated by a gradual decline in the median values as shown in Table 8. However, no patients receiving three or more courses at any dose level experienced any complications as a result of the leukopenia and/or thrombocytopenia. This suggests that CZT like other chloroethyl nitrosoureas may produce progressive myelosuppression but perhaps to a lesser degree.

DISCUSSION

A phase II extensive clinical trial of CZT in human malignancies has been disappointing in that the objective responses observed have been limited in this study with the drug doses employed. However, in two categories, lymphoma and sarcoma, objective responses were observed. In both types of neoplasia all of the patients had received fairly extensive prior chemotherapy. Further evaluation of CZT in colorectal, breast and non-oat cell carcinoma of the lung by this method of administration does not appear warranted. One of the initial phase I studies [5] suggested responses in 3 kidney carcinomas; however, a recent report by Gralla [6] in which no responses were seen in 21 patients, plus the 9 patients treated in this study, would suggest that further exploration of CZT in this disease category would be of no value. Perhaps additional patients will be needed to adequately evaluate CZT in melanomas and pancreatic and gastric cancers. Also, perhaps higher initial doses of CZT might be considered as well as different schedules of administration to determine if the efficacy of CZT might be improved.

The initial premise that the glucose carrier of a chloroethyl nitrosourea should have less hematologic toxicity appears to be supported by the results of this study. However, the hematologic toxicity of CZT appears to be cumulative though less so than other chloroethyl nitrosoureas.

Apparently by decreasing the carbamoylating activity of CZT as compared to other chloroethyl nitrosoureas, this has occurred. However, there apparently has been a decrease in the response rates one might expect in tumors such as breast, colorectal, lung, and perhaps melanoma, suggesting that the carbamoylating activity may be important in the activities of the other chloroethyl nitrosoureas.

Hepatic and renal toxicities occurred in a small number of patients, but were not as severe as was reported in preclinical animal toxicity studies [7]. In all instances, the mild to moderate renal and hepatic biochemical abnormalities were reversible. The one patient with severe azotemia did not experience a return to normal values of the BUN and creatinine.

CONCLUSION

Chlorozotocin in an initial dose of 90–120 mg/m^2 is relatively non-myelosuppressive. Significant antitumor effectiveness has been limited in this study to lymphomas and sarcomas. Whether or not initial higher doses of the chlorozotocin to determine response should be employed is currently under study by other investigators but the results have not been reported. Inclusion of CZT in combination therapy for these neoplasms should be considered because of the activity demonstrated and the relative non-hematologic toxicity of CZT. However, this study was not designed to determine a dose of chlorozotocin to be employed with other cytotoxic agents. Further study of chlorozotocin should include higher initial doses, different dosage regimens and phase I studies in combination with other chemotherapeutic agents.

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