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Clinical Pharmacology of the Anticancer Polypeptide Neocarzinostatin

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Summary. *The clinical pharmacology of the anticancer polypeptide neocarzinostatin was studied in 16 patients with disseminated neoplasia using a radioimmunoassay technique. Patients who received 2,400–3,600 U of the drug per square meter BSA by rapid IV infusion had triphasic plasma decay curves. For eight patients with normal hepatic and renal function, neocarzinostatin mean plasma half-lives were 0.14, 0.50, and 7.7 h. The mean plasma drug clearance was 32.4 ml/min/m² and the apparent volume of distribution 19.3 l/m². Two patients with liver dysfunction had shorter terminal plasma half-lives and greater drug clearances, while two with renal disease exhibited prolonged plasma half-lives and reduced drug clearances. The mean cumulative urinary excretion of neocarzinostatin was 69.1% of the administered dose at 72 h in three patients with normal hepatic and renal function. One patient with liver disease excreted 90.4%, while a patient with renal disease excreted only 58.1% of the dose in 24 h. In one patient with marked liver disease, biliary excretion accounted for 0.1% of the administered dose in 72 h. Cerebrospinal fluid concentrations of neocarzinostatin studied in two patients showed a CSF penetration of about 16% the plasma concentration at 1–5 h; concentrations persisted for 19 h in one patient with an Omayha reservoir. Neocarzinostatin was rapidly cleared from the plasma and eliminated in the urine. Dosage reductions of 50% are recommended for patients with impaired renal function, while no reduction or escalated doses could be tolerated by patients with liver disease. The pharmacologic data suggest a continuous IV infusion may be a more toxic but perhaps more effective schedule of administration.*

Introduction

Neocarzinostatin is an acidic, highly folded, single-chain polypeptide extracted from *Streptomyces carzinostaticus var. F41* by Japanese investigators [7]. The drug contains 109 amino acid residues and has a molecular weight of about 9,000 [12]. In *Sarcina lutea,* HeLa cells, and Burkitt's lymphoma cells neocarzinostatin has been shown to affect DNA more profoundly than RNA or protein synthesis, with single- and double-strand DNA breaks observed [8, 10, 14]. The drug affects cells primarily in the G-2 phase of the cell cycle, with less effective but cytocidal activity against cells in G-1 and M phases [1, 15, 17].

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Anticancer effects of neocarzinostatin have been documented in mice and man. Activity has been shown against ascitic sarcoma 180, ascitic leukemia SN-36, and L1210 leukemia in mice [1, 17]. Most clinical trials in man have been conducted in Japan, where antitumor responses have been reported in acute leukemia and solid tumors of the rectum, stomach, bladder, and penis (M. Slavik, personal communication). In a phase I clinical trial at our Institution, neocarzinostatin has shown activity in acute and chronic leukemia [13]. Myelosuppression has been the dose-limiting toxicity, with nausea, chills, fever, and mucositis other observed side-effects.

Concomitant with our phase I clinical trial of neocarzinostatin, we studied the drug's pharmacokinetics. Antibodies directed against neocarzinostatin have been produced and form the basis from which a very sensitive radioimmunoassay has been developed [16]. With a double-antibody technique, Samy and Raso were able to detect antibody bound and free antigen at a concentration of 1×10^{-13} mole [16]. Preliminary pharmacokinetics of neocarzinostatin in the rat demonstrated that the drug rapidly disappeared from the plasma, 90% being removed within 10 min of injection. The studies, however, did suggest a second longer plasma half-life. We performed pharmacokinetic studies of neocarzinostatin in man utilizing a radioimmunoassay technique to detect drug in biological fluids. The drug's clinical pharmacology was studied to aid in determining the most effective schedule for drug administration.

Patients and Methods

There were 18 plasma pharmacokinetic studies of neocarzinostatin performed in 13 patients. The patients' clinical characteristics are shown in Table 1. All patients were participants in the phase I clinical trial of the drug and gave written informed consent according to institutional policy prior to receiving the drug.

Of the 13 patients studied, eight were considered to have normal liver and renal function (nos 1–8, Table 1). Two patients had markedly abnormal liver function related to extensive hepatic involvement with carcinoma (nos 9 and 10), and two patients were considered to have abnormal renal function as evidenced by elevated serum creatinine concentrations (nos 11 and 12). One additional patient had both impaired liver and renal function (no 13). In addition to plasma pharmacology, two patients had cerebrospinal fluid (CSF) concentrations determined (nos 1 and 6) and one patient with a

Table 1. Patients' clinical characteristics

Patient no.	Age (years)	Sex	Diagnosis	Day RX studied	Dose (U/m^2)	Liver function[a]			Renal function	
						Bil	SGOT	Alk Phos	Bun	Cr
1	45	M	Acute myelocytic leukemia	12	3,600	0.6	8	55	12	1.0
				14	3,600	0.6	8	55		
2	55	M	Lymphoma	1	3,600	0.3	30	80	9	1.1
				5	3,600	0.3	30	80		
3	64	M	Prostate carcinoma	1	2,400	0.5	24	350	25	1.1
				5	2,400	0.5	24	350		
4	65	M	Adenocarcinoma (primary?)	5	3,600	0.3	40	151	11	0.7
5	59	M	Mycosis fungoides	5	3,600	0.3	30	116	17	1.2
6	42	F	Breast carcinoma	1	2,500	0.5	30	88	5	0.6
7	61	M	Chronic myelocytic leukemia	1	3,600	0.6	19	91	10	1.0
8	53	M	Chronic lymphocytic leukemia	1	2,400	0.5	16	51	26	1.2
9	16	F	Hepatoma	1	3,600	0.5	29	997	10	0.5
				3	3,600	0.5	29	997		
10	57	M	Pancreatic carcinoma	1	3,600	7.0	95	350	13	0.8
				5	3,600	7.0	95	350		
11	77	M	Esophagus squamous cell	1	3,600	0.8	21	73	30	1.7
12	46	M	Renal carcinoma	5	2,700	0.8	20	91	31	2.1
13	40	M	Hepatoma	1	3,600	10.0	300	360	27	2.0

[a] Normal laboratory values are: Bilirubin 0.15–01.0 mg/100 ml; SGOT 10–50 mu/ml; Alk Phos 30–85 mu/ml; BUN 10–20 mg/100 ml; Creatinine 0.7–113 mg/100 ml

Table 2. Neocarzinostatin pharmacokinetics[a]

Organ status	No. of studies/ no. of patients	$t_{1/2}$ (h)	$t_{1/2}$ (h)	$t_{1/2}$ (h)	Clearance ($ml/min/m^2$)	VD (l/m^2)
Normal	11/8	0.14 ± 0.02	0.50 ± 0.13	7.74 ± 1.29	32.34 ± 4.56	19.28 ± 2.86
Abnormal liver	2/1 (no. 10)[b]	0.26	0.48	5.69	41.46	20.41
		0.22	0.73	7.74	27.97	18.75
Abnormal liver	2/1 (no. 9)	0.04	0.30	5.88	73.80	37.61
		0.47	0.42	3.81	52.34	17.28
Abnormal liver	1/1 (no. 12)	0.36	0.71	3.61	17.51	54.67
Abnormal kidney	1/1 (no. 11)	0.16	2.21	11.73	14.18	14.39
Abnormal liver and kidney	1/1 (no. 13)	0.18	0.92	10.93	12.15	11.50

[a] Values are means ± SE
[b] Relates to patient number in Table 1

Table 3. Neocarzinostatin C × t related to dose

Dose ($units/m^2$)	Organ status	No. of studies	No. of patients	C × t mU-h/ml
2,400	Normal	3	2	1.583 ± 1.648[a]
2,500	Normal	1	1	2.566
2,700	Abnormal kidney	1	1	2.570
3,600	Normal	7	5	2.185 ± 0.356
3,600	Abnormal liver	4	2	1.388 ± 0.284
3,600	Abnormal kidney	1	1	4.233
3,600	Abnormal liver and kidney	1	1	4.937

[a] Values are means ± SE

bile diversion system had two studies of the biliary elimination of neocarzinostatin (no 10).

All patients received neocarzinostatin, in doses ranging from 2,400–3,600 U/m^2/day for 5–10 days. The drug was diluted in 50–100 ml 5% dextrose solution and infused as rapidly as possible, usually over 5–10 min. Blood, bile, and urine samples were obtained before treatment and serially afterward; the blood was placed in heparinized tubes, centrifuged and the plasma separated. Bile was collected serially as it drained from the biliary diversion system. Spinal taps were performed at selected times after drug administration and CSF collected. Plasma, urine, bile, and CSF were frozen for later analysis.

Neocarzinostatin concentrations were determined by radioimmunoassay. The radioimmunoassay procedure was a competitive protein-binding assay between ^{125}I-labeled neocarzinostatin and unlabeled neocarzinostatin for binding sites. The preparation of the neocarzinostatin-bovine serum albumin conjugate and the immunization of New Zealand White rabbits to produce the antibody and the neocarzinostatin iodination using a modification of the Hunter and Greenwood chloramine T technique were similar to the procedures reported for bleomycin [2]. The neocarzinostatin radioimmunoassay standard curve was linear on a logit-log plot with the lower limit of sensitivity less than 1 ng/ml or less than 0.002 U/ml.

The plasma pharmacokinetic data appeared to best fit a triphasic plasma decay curve. Pharmacokinetic calculations were done utilizing best curve fitting by nonlinear least-squares regression analysis.

Results

The plasma pharmacokinetics of neocarzinostatin are summarized in Table 2. Eleven studies were performed in eight patients with normal liver and renal function with a mean initial half-life ($t_{1/2}$) of 0.14 h, a second $t_{1/2}$ of 0.50 h, and a terminal $t_{1/2}$ of 7.74 h. The mean clearance of drug in these patients was 32.4 ml/min/m^2, with a volume of distribution (Vd) of 19.3 l/m^2. The mean plasma disappearance curve for patients with normal organ function is shown in Fig. 1.

Seven plasma pharmacokinetic studies were done in five patients with abnormal liver and/or renal function. These results are also seen in Table 2. In the two patients with liver disease there was a slight decrease in plasma drug disappearance, as evidenced by a decreased $t_{1/2}$, and a suggestion of a greater drug clearance. An example of the plasma disappearance of neocarzinostatin in one of the patients with liver disease is shown in Fig. 1. Although there were only two patients with liver disease, there is a suggestion that these patients had an enhanced plasma clearance of neocarzinostatin compared with patients who had normal hepatic function.

Two patients with significant renal dysfunction were studied and their neocarzinostatin plasma pharmacokinetics are shown in Table 2, with a representative plasma disappearance curve illustrated in Fig. 1. The $t_{1/2}$ in these patients was 3.6 and 11.7 h, as against 7.7 h for normal patients. However, the plasma clearances for the two patients with renal disease were reduced by about 50%, to 17.5 and 14.2 ml/min/m^2, as against 32.4 ml/min/m^2 for normal patients. It appears that renal disease significantly retards the plasma clearance of neocarzinostatin. One patient with both liver and renal disease (Table 2, Fig. 1) had a $t_{1/2}$ prolonged to 10.9 h and the lowest plasma clearance of any patient studied (12.2 ml/min/m^2). These data suggest that the retardation of plasma drug clearance that results from renal disease appears more important than the enhanced drug clearance seen in patients with liver dysfunction.

The area under the neocarzinostatin plasma decay curve (C × t) is compared with the administered dose in Table 3. For three studies in two patients with normal organ function who received 2,400 U neocarzinostatin/m^2, the C × t was about 1.58 mU-h/ml and for seven studies in five patients who received 3,600 U/m^2, was 2.18 mU-h/ml. At the 3,600 U/m^2 dose, the mean C × t was reduced to approximately 1.39 mU-h/ml in patients with liver disease. This was less than the

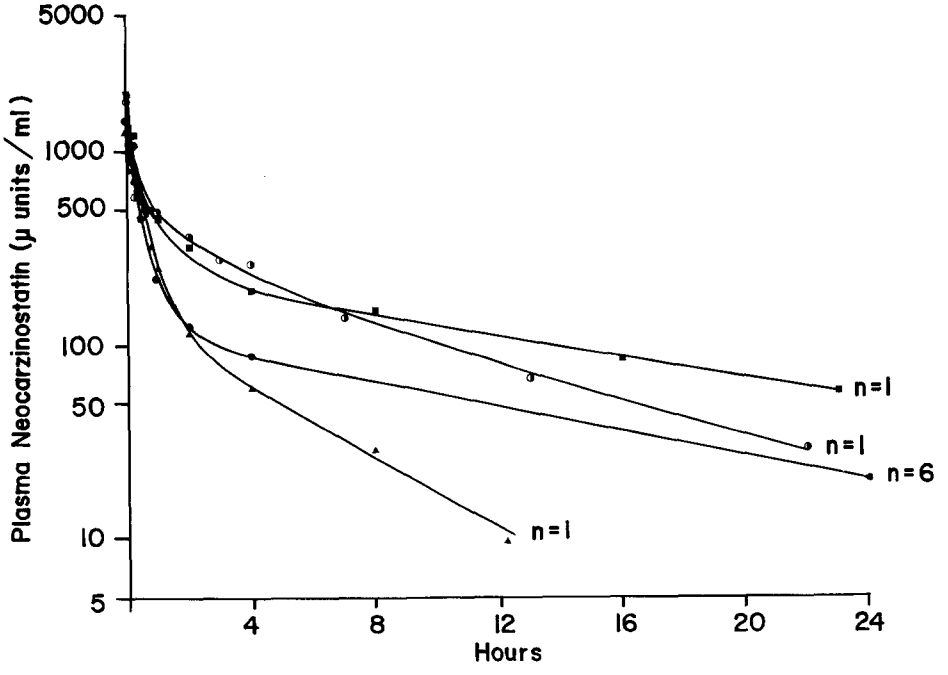

Fig. 1. The mean plasma disappearance curve for neocarzinostatin in six patients with normal hepatic and renal function (●) and representative plasma neocarzinostatin disappearance curves in one patient with liver disease (▲), one with renal disease (◑), and one with both renal and hepatic disease (■). All patients received 3,600 U neocarzinostatin/m^2 IV

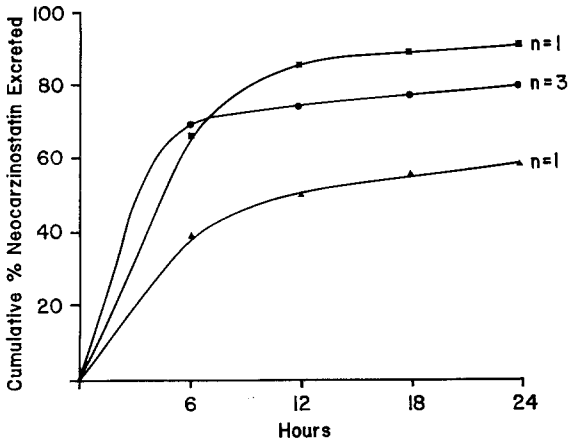

Fig. 2. The mean % cumulative urinary excretion of neocarzinostatin in three patients with normal hepatic and renal function (●), and representative cumulative urinary excretion curves for one patient with hepatic disease (■) and one with renal disease (▲)

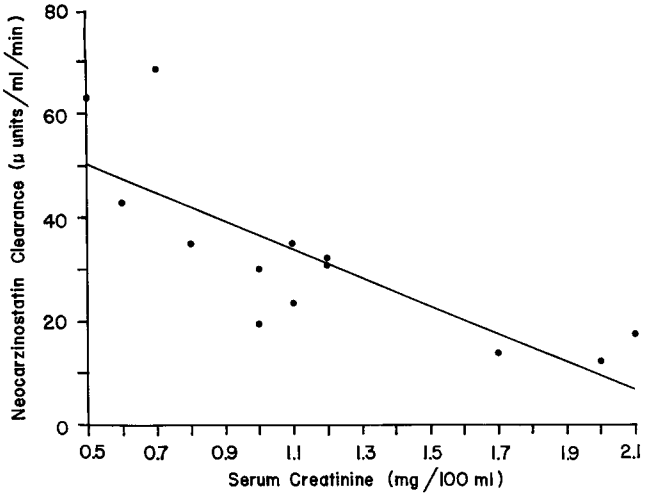

Fig. 3. Neocarzinostatin plasma drug clearance versus serum creatinine concentration in 13 patients ($r^2 = 0.61$)

Table 4. Neocarzinostatin cumulative % biliary excretion in a patient with liver disease

Time[a]	Day 1	Day 5
0–6	0.03	0.01
6–12	0.04	0.02
12–18	0.05	0.02
18–24	0.07	0.02
24–48	–	0.04
48–72	–	0.04

[a] Hours after drug administration

Table 5. Neocarzinostatin CSF pharmacokinetics

Patient	Time[a]	Plasma concentration (μU/ml)	CSF concentration (μU/ml)	CSF/plasma (%)
1	0	1,124	1	0.09
	1 1/3	100	5.2	5.2
	2	63.8	5.7	8.9
	4	39.7	6.6	16.6
	19 1/4	9.5	5.9	62.1
2	7.5	89.0	8.0	9.0

[a] Hours after drug administration

C × t for normal patients who received 2,400 U/m^2 or 50% less drug. The two patients with renal disease, as expected, had increased C × t values compared with normal patients who received comparable doses. This was predictable from their decreased drug clearance.

The urinary excretion of neocarzinostatin was studied serially in five patients with the cumulative percent of the administered dose excreted shown in Fig. 1. For three patients with normal renal and hepatic function 69.1% ± 1.4% (SE) was excreted in 6 h, with an additional 10% excreted over the next 18 h or 79.0% ± 4.0% (SE) of the administered dose recovered from the urine in 24 h. One patient with liver disease excreted 90.4% of the administered dose in 24 h, while another with renal disease excreted only 58.1% of the dose by 24 h. These data show that neocarzinostatin is rapidly excreted in the urine and patients with liver disease excrete increased amounts of the administered dose while patients with renal disease excrete less. Figure 3 shows the total plasma clearance of neocarzinostatin related to serum creatinine concentrations in our 13 patients. The patients with the lower serum creatinine concentrations had the greatest drug clearance, while those with elevated serum creatinine concentrations had the lowest clearances. The least-square regression analysis of this curve had an r^2 of 0.61, which suggests a correlation between total plasma clearance and serum creatinine concentration. Obviously the correlation between neocarzinostatin renal clearance and serum creatinine concentration would be more striking.

One patient with liver disease (no 10, Table 1) had bile collected via a percutaneous biliary fistula placed for the treatment of obstructive jaundice due to tumor. The patient received 3,000 U neocarzinostatin/m^2 on days 1–5, with bile collections for drug determinations done on days 1 and 5. The results are shown in Table 4. Biliary excretion accounted for < 0.1% of the administered dose up to 72 h after drug administration. This patient had marked impairment of hepatic function (serum bilirubin concentration of 7.0 mg/dl), which could account for the decreased biliary excretion of neocarzinostatin. The low amount recovered, however, suggests the biliary route of drug elimination may not be a major one.

Two patients (nos 1 and 6, Table 1) had CSF concentrations of neocarzinostatin determined and these are compared with simultaneously obtained plasma levels of the drug in Table 5. Peak CSF concentrations were obtained 4–7.5 h after drug administration and were 9%–17% of the plasma concentrations. In one patient who had multiple samples obtained, the CSF neocarzinostatin concentration increased to 5.2 μU/ml. The persistence of neocarzinostatin in the CSF suggests extremely slow elimination of the drug from the CSF, despite the low drug concentrations obtained.

Discussion

Our clinical pharmacology studies of neocarzinostatin by radioimmunoassay show the drug to have a triphasic plasma disappearance. The pharmacology of neocarzinostatin reported by Comis et al. [3] showed a rapid biphasic plasma clearance with a second phase $t_{1/2}$ ranging from 25 to 231 min. They could not detect serum levels beyond 12 h. We were able to detect levels over a range of 20–100 μU/ml plasma in most of our patients at 24 h, and to show what we feel is a third phase to the plasma disappearance. How clinically or pharmacologically important this phase is has not been determined. The mean terminal $t_{1/2}$ was 7.7 h, and this is still short enough to suggest that other schedules of administration, such as a continuous IV infusion, may be effective.

The drug's plasma disappearance is most affected by its urinary excretion. Consistent with the predominant urinary excretion of the drug, patients with renal disease have a decreased drug urinary excretion and plasma clearance while patients with liver disease have an increased urinary excretion and plasma clearance. This suggests the drug may be sequestered in, bound to, or metabolized by the liver in patients with normal liver function. Patients with liver disease have a reduction in this hepatic process with greater urinary excretion of the drug. Studies of neocarzinostatin in rodents with L1210 and P388 tumors showed the liver and gastrointestinal tract to retain drug at 24 h suggesting another route of elimination [11].

Neocarzinostatin penetrates the CSF poorly but appears to be slowly eliminated from this space. Studies in tumor-bearing animals have also showed poor uptake and little accumulation of neocarzinostatin in brain tissue [11].

The pharmacokinetics of neocarzinostatin is similar to bleomycin, another anticancer polypeptide which has also been studied by radioimmunoassay. Bleomycin also has a short elimination $t_{1/2}$ with predominant urinary excretion [5, 9]. The drugs have other similarities with activity in lymphoid neoplasms and mechanisms of action that cause scissions in DNA strands.

The specificity of the radioimmunoassay for neocarzinostatin has not been extensively studied, nor are there studies showing extensive metabolism of the drug. Our technique may be measuring drug fragments or metabolites in addition to unchanged neocarzinostatin, and further studies are needed to confirm the specificity of the assay and determine whether or not the drug is significantly metabolized or degraded in vivo.

The pharmacology of neocarzinostatin suggests some clinical maneuvers that might optimize its anticancer effects. Drug dosages should be reduced for patients with significant renal disease. We suggest a reduction of about 50% for patients with serum creatinine concentrations of 1.8–2.0 mg/dl. This reduction would be most important for solid tumor patients who need to avoid the myelosuppressive toxicity of the drug. The pharmacologic data suggests that patients with liver disease could tolerate dose escalations; starting doses of 33%–50% higher than those given to patients with normal liver function should be considered for these patients.

Because of the rapid elimination of neocarzinostatin, continuous IV infusion of the drug may be of advantage. A continuous infusion would result in a higher C × t and perhaps greater cell kill. There are preliminary data suggesting this schedule is advantageous in patients with acute leukemia [4]. Additionally, with the clinical activity of the drug in leukemia and its very slow elimination from the CSF, high-dose IV or intrathecal neocarzinostatin therapy for patients with meningeal leukemia would be of interest.

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