Pharmacokinetic Analysis of Neocarzinostatin in Normal and Tumor-bearing Rodents[1]

Ivan S. Lowenthal,[2] Leroy M. Parker, David J. Greenblatt,[3] Barbara L. Brown, and T. S. Anantha Samy[4]

Sidney Farber Cancer Institute [I.S.L., L.M.P., B.L.B., T.S.A.S.] and Clinical Pharmacology Unit, Massachusetts General Hospital [D.J.G.], Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT

Neocarzinostatin (NCS) is an acidic protein with proven antitumor activity in experimental animals and is now in clinical trial as a cancer chemotherapeutic agent. Utilizing a ^{125}I-labeled bisamino-modified derivative, the distribution, excretion, and metabolism of NCS have been studied in normal and tumor-bearing rodents.

NCS is rapidly excreted in the urine, with about 55% of the drug excreted unchanged in the first hr. About 10% of the radioactivity remained in the blood 1 hr after administration and was associated with peptides with molecular weights of ~1500; these were not detected in the urine. The mean urinary excretion half-life of 1.65 hr was less than the apparent elimination half-life from blood (6.2 hr). Biliary excretion contributed little to the total clearance of NCS, since only a small fraction of the drug appeared unchanged in the bile. Although biliary excretion increased in the anephric rat, it proceeded at a maximum rate of 0.25 to 0.3%/hr. Tissue levels of ^{125}I-NCS were highest in kidney, the principal organ of excretion. Decreasing levels were observed in the skin, lung, intestine, pancreas, liver, spleen, muscle, thymus, and bone. ^{125}I-NCS was low in the testis and practically excluded from the brain. Radioactivity due to ^{125}I-NCS persisted in the Walker 256 ascites tumor in the rat but not in the ascites of the L1210 leukemia in mice. Since there is rapid elimination of the drug from the central compartment and little persistence in the tissues 6 hr after single (i.v.) injection, optimal scheduling may be frequent single injections or short continuous-flow infusions.

INTRODUCTION

NCS,[5] an acidic antibiotic protein isolated from *Streptomyces carzinostaticus*, is active in the treatment of transplantable rodent tumors (1, 9, 17) and of solid tumors (6, 20) and leukemias in humans (8, 14). During investigations on the relationship of NCS structure to biological activity, we obtained a bisamino-modified derivative by reaction of the 2 amino groups of NCS with 3-(4-hydroxyphenyl)propionic acid N-hydroxysuccinimide ester. The purified bisamino derivative retained its antibiotic and antitumor effects (16, 18) as well as cross-reactivity towards antiserum to native NCS (19). By means of a radiolabeled acylating agent, ^{125}I-NCS was pre-

pared. We have used ^{125}I-NCS to study the distribution, excretion, and metabolism of NCS in normal and tumor-bearing rodents.

MATERIALS AND METHODS

Analytical-grade chemicals were used in this study. NCS, either as a crude preparation of the culture filtrate or the clinical formulation (Kayaku Antibiotics, Ltd., Kyoto, Japan), was purified to homogeneity, according to a previously published procedure (17). Bis-HPP-NCS was prepared by reaction of the protein with 3-(4-hydroxyphenyl)propionic acid N-hydroxysuccinimide ester in 0.1 M borate buffer, pH 8.5, and was purified by chromatography of the reacted protein through Sephadex G-25 and carboxymethylcellulose columns. The purified bis-HPP-NCS was biologically active as demonstrated by its inhibition of growth of *Sarcina lutea* and CCRF-CEM leukemic cells *in vitro* and by its antitumor activity equivalent to NCS in C57BL × DBA/2 F$_1$ (hereafter called BD2F$_1$) mice bearing P388 and L1210 leukemia (16, 18). Details of its immunoreactivity have also been published (19).

^{125}I-NCS (specific activity, 30 to 40 Ci/mmol) was prepared by the same procedure utilizing 3-(3-^{125}I,4-hydroxyphenyl)propionic acid N-hydroxysuccinimide ester [Bolton-Hunter reagent (2); specific activity, 1700 Ci/mmol; Amersham/Searle, Arlington Heights, Ill.].

Normal BD2F$_1$ mice (average weight, 20 g) and Sprague-Dawley rats (average weight, 250 g) were purchased from The Jackson Laboratory, Bar Harbor, Maine, and Charles River Breeding Laboratories, Wilmington, Mass., respectively. The experiments with L1210 leukemia in mice are carried out routinely in our laboratories. Rats bearing i.m. or i.p. implanted Walker 256 tumors were obtained from I. Wodinsky, Arthur D. Little, Inc., Cambridge, Mass. L1210 leukemic cells (10^5 cells/ mouse) were inoculated i.p. 4 days prior to drug distribution experiments. Normal and tumor-bearing animals received a mixture of bis-HPP-NCS at a dose of 2 to 3 mg/kg containing approximately 0.5 to 2 μCi of ^{125}I-NCS. At selected time intervals, venous blood was sampled from the retroorbital plexus, and bile and urine samples were obtained from the previously cannulated common bile duct and catheterized bladder. At intervals, mice and rats were sacrificed by either cervical dislocation or exsanguination. Tissues were dissected, rinsed in 0.9% NaCl solution, blotted to remove excess fluid, and weighed. The radioactivity of the samples was determined using a Packard Auto-Gamma spectrometer. Results were expressed both as μg of drug per mg wet weight or per ml and as a percentage of the total administered dose.

Urine, bile, and blood samples were analyzed by chromatography of the sample on a Sephadex G-15 column (1.5 × 90 cm). The column was eluted with 0.2 M sodium chloride, and

3.7-ml fractions were collected. Insulin, adrenocorticotropic hormone, and actinomycin D were used to calibrate the column for determination of the molecular weight of the eluting peptides. Recovery of the radioactivity from the column was 80% for bile, 86% for urine, and 95% for blood.

Blood concentrations of NCS were fitted by computer using weighted nonlinear least-squares regression analysis (13, 22) to the function:

$$C = A e^{-\alpha t} + P e^{-\pi t} + B e^{-\beta t} \qquad \text{(A)}$$

where C is the blood concentration and t is the time after dose. Equation A is consistent with a 3-compartment open pharmacokinetic model (4, 23) but is not specific for a particular configuration of compartments. A, P, and B are hybrid coefficients; α, π, and β are hybrid exponents (5). Based upon standard pharmacokinetic methods (4, 5, 23), these coefficients and exponents for individual animal data and for the composite data points (based on the mean NCS concentrations from different animals at corresponding points in time) were used to calculate the initial distribution half-life ($t_{1/2}\,\alpha$), intermediate distribution half-life ($t_{1/2}\,\pi$), elimination half-life ($t_{1/2}\,\beta$), apparent volume of the central compartment (V_1), total volume of distribution by the area method (V_d), and total plasma clearance. The fitted functions were also used to predict the distribution of the drug between the central tissue compartment and the amount eliminated (15).

Apparent half-lives for urinary excretion were obtained by least-squares regression analysis, using the terminal log-linear portion of graphs of excretion rate versus the midpoint of the collection interval (4, 23).

RESULTS

Preliminary drug distribution and excretion studies were carried out in normal and L1210-bearing mice. Sixty min following a 2-mg/kg i.v. injection containing ^{125}I-NCS, drug concentrations varied over 3 orders of magnitude in tissues. The highest concentrations were present in the kidney, and the lowest concentrations were found in the brain (Table 1). Testes and bone also had low concentrations, while levels in ascites, lung, liver, and intestines were similar to those in blood. Urine, within the bladder, accounted for 45% of the administered dose but was present at a NCS concentration of 13 μg/ml. This was the highest concentration of NCS found at the 60-min time point.

At 24 hr, the highest drug concentrations were noted in kidney and intestine, reflecting those routes of excretion. Feces collected over a 24-hr period contained only 3.5% of the administered dose. Lower concentrations at 24 hr were noted in brain and testes. Twenty-four hr after drug administration, the weights of the spleen and thymus glands of mice receiving bisamino-substituted NCS (2 mg/kg) fell from 144 ± 3 (S.E.) and 36 ± 0.75 to 75 and 20 mg, respectively. This lympholytic effect is similar to that produced by native NCS. No accumulation of NCS in ascites was observed. Drug distribution following i.v. administration was not influenced by the presence of ascitic L1210 tumor.

The concentration of NCS in rat blood decayed as a triexponential function with time (Chart 1). The half-lives for each exponential phase are given in Table 2. The initial phase had

Table 1
Tissue concentration of ^{125}I-NCS in mice after single i.v. injection

| Tissue | NCS concentration (μg/g) | |
	60 min	24 hr
Kidney	3.0 ± 0.9[a]	0.77 ± 0.13
L1210 ascites	0.3 ± 0.04	0.004 ± 0.002
Lung	0.47 ± 0.13	0.014 ± 0.003
Intestine	0.41 ± 0.04	0.041 ± 0.010
Blood	0.63 ± 0.28	0.011 ± 0.003
Liver	0.45 ± 0.07	0.010 ± 0.001
Salivary gland	0.27 ± 0.04	0.010 ± 0.001
Spleen	0.22 ± 0.04	0.009 ± 0.001
Thymus	0.17 ± 0.04	0.016 ± 0.004
Bone	0.14 ± 0.01	0.012 ± 0.002
Testis	0.060 ± 0.02	0.002 ± 0.005
Brain	0.030 ± 0.001	0.001 ± 0

[a] Mean ± S.E. ($n \geq 3$) after 2-mg/kg dose.
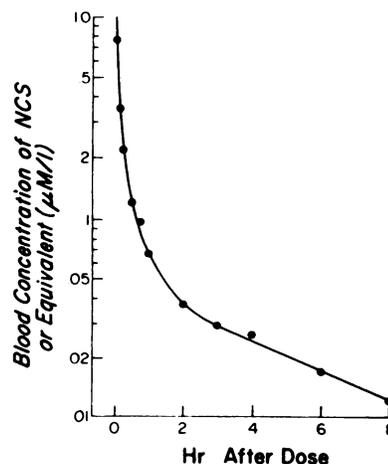
Chart 1. Concentration of total ^{125}I in blood expressed as concentration of NCS during 8 hr following single i.v. 3-mg/kg injection. Each point is the mean concentration (± S.E.) for 4 animals at corresponding times. ——, computer-determined pharmacokinetic function. $\mu M/l$, μmol/liter.

Table 2
Pharmacokinetic parameters for ^{125}I-NCS in rats after single i.v. injection

| Animal | Half-life (hr) | | | V_1 (liters/kg) | V_d (liters/kg) | Total clearance (ml/min/kg) | Total clearance (ml/min/kg) |
	$t_{1/2}\,\alpha$	$t_{1/2}\,\pi$	$t_{1/2}\,\beta$				
1	0.03	0.73	6.8	0.15	4.98	8.46	6.49
2	0.16		2.6	0.68	4.22	18.73	14.29
3	0.04	0.58	5.8	0.09	4.29	8.53	5.88
4	0.05	0.34	9.4	0.11	3.91	4.82	2.71
Mean ± S.E.	0.07 ± 0.03	0.55 ± 0.11	6.2 ± 1.4	0.26 ± 0.14	4.35 ± 0.23	10.14 ± 3.00	7.34 ± 2.46
Composite	0.04	0.34	4.1	0.13	3.21	9.11	

a mean half-life of 0.07 ± 0.03 (S.E.) hr, followed by a slower phase with half-life of 0.55 ± 0.11 hr and a final phase with a half-life of 6.2 ± 1.4 hr. The apparent volume of the central compartment (V_1) averaged 0.26 liter/kg, while the total volume of distribution V_d was 4.35 ± 0.23 liter/kg.

Most rat organ tissue concentrations became similar to the blood NCS concentration by 45 min after injection, including skin and muscle, in which there is relatively low blood flow (Table 3). Exceptions were brain and testes as previously noted in the mouse. By 6 hr, only kidney and intestines had cytotoxic concentrations of NCS remaining.

The kinetics of drug uptake and elimination in the i.m. Walker tumor was similar to that of other rat tissues. However, NCS persisted in Walker ascites with a significant concentration of drug still apparent at 6 hr. In neither case did the presence of tumor affect drug uptake, distribution, or elimination in other tissue compartments.

NCS was rapidly excreted in urine, with about 55% of the dose excreted in the first hr. By 8 hr, total urinary excretion accounted for 70% of the administered dose (Table 4). The total clearance of NCS (10.1 ± 3.0 ml/min/kg) multiplied by the fraction of drug excreted in the urine at 8 hr was 7.34 ± 2.46 ml/min/kg which is an estimate of renal clearance. The mean urinary excretion half-life was 1.65 ± 0.19 hr (Table 4), which is similar to the value (1.51 hr) calculated from the composite curve for the urinary excretion rate (Chart 2). Both these values are substantially less than the apparent terminal half-life of NCS disappearance from the blood [$t_{1/2} \beta$ = 6.2 hr (Table 2)].

Table 3
Tissue concentration of ^{125}I-NCS in rats after single i.v. injection

Tissue	NCS concentration (μg/g)	
	45 min	6 hr
Kidney	16 ± 1[a]	1.1 ± 1
Walker ascites	1.6 ± 0.1	0.12 ± 0.01
Skin	1.4 ± 0.05	0.039 ± 0.008
Lung	0.95 ± 0.10	0.037 ± 0.006
Intestine	0.91 ± 0.20	0.04 ± 0.12
Blood	0.82 ± 0.04	0.16 ± 0.03
Pancreas	0.80 ± 0.04	0.034 ± 0.005
Liver	0.60 ± 0.05	0.039 ± 0.004
Walker tumor (i.m.)	0.58 ± 0.02	0.038 ± 0.004
Spleen	0.52 ± 0.03	0.036 ± 0.004
Muscle	0.46 ± 0.04	0.000 ± 0.002
Testis	0.34 ± 0.007	0.000 ± 0.002
Brain	0.03	<0.010

[a] Mean ± S.E. (n = 4) after 3-mg/kg dose.

Table 4
Excretion of ^{125}I-NCS in the urine and bile during 8 hr following single i.v. injection

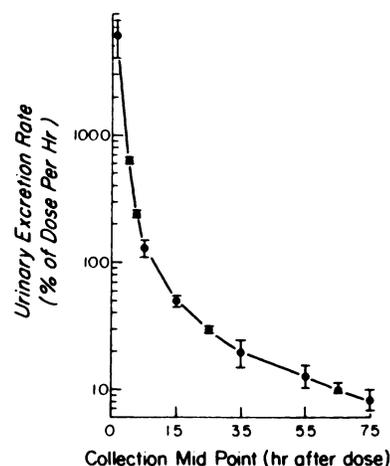|	Total radioactivity		
	Urine		
Animal	Cumulative % of dose	Excretion half-life (hr)	Bile (cumulative % of dose)
1	76.7	1.82	1.02
2	76.3	1.82	0.93
3	70.2	1.08	1.03
4	58.1	1.89	0.96
Mean ± S.E.	70.3 ± 4.3	1.65 ± 0.19	0.99 ± 0.02
Composite		1.51	1.02

Chart 2. Mean urinary excretion rates of ^{125}I-NCS (n = 4), as percentage of the administered dose per hr, during 8 hr following single i.v. injection. *Bars*, S.E.

Table 5
Pharmacokinetic parameters for ^{125}I-NCS in anephric rats after single i.v. injection

Animal	$t_{1/2} \alpha$ (hr)	$t_{1/2} \beta$ (hr)	V_1 (liters/kg)	V_d (area) (liters/kg)	Total clearance (ml/min/kg)
1	0.21	82.7	0.11	0.47	0.07
2	0.05	6.7	0.05	0.24	0.42
3	0.05	18.8	0.07	0.35	0.21
Mean ± S.E.	0.10 ± 0.05	36.1 ± 23.6			

As in the mouse, bile excretion in the normal rat contributed little to the total clearance of NCS (Table 5). The contribution of this route of excretion in renal insufficiency was studied by ligating both ureters prior to single i.v. administration. The pharmacokinetic data for the functionally anephric state are presented in Table 5. The decay of blood ^{125}I-NCS concentration was consistent with a biexponential function with a prolonged elimination phase of 36.1 ± 23.6 hr. The large standard error may reflect variability in residual renal function after ligation. The ratio of V_d to V_1 was reduced from 17:1 in the intat animal to 5:1 in the functionally anephric animal. Total clearance was decreased to less than 5%. The amount of ^{125}I-NCS appearing in bile was increased (Chart 3) with excretion proceeding as an apparent zero-order process at a rate of 0.25 to 0.3%/hr.

^{125}I-NCS isolated from urine was unchanged as determined by gel filtration chromatography in comparison with the administered drug (Chart 4). In order to test whether the NCS excreted in the urine had retained biological potency, 2 normal rats were given a single dose of NCS, 2 mg/kg i.v., and urine was collected by catheterization at the end of 1 hr. The concentration of NCS excreted was determined to be 37.2 μg/ml by radioimmunoassay. The urine was assayed for *in vitro* growth-inhibitory activity against *S. lutea* and human leukemic (CCRF-CEM) cells in suspension culture. The 50% inhibitory dose was equivalent to that of administered native NCS (0.035 μg/ml). These data suggested that most administered NCS is excreted into the urine unchanged, as judged by chromatography on Sephadex G-25, and is biologically active.

In contrast, 25 to 35% of the radioactivity in the blood at 1 hr and more than 50% of the radioactivity in the bile at 8 hr were associated with peptides with molecular weights of 1500 and below (Chart 4). In blood, this represents less than 1% of the administered dose, which may account for the difficulty in detecting low-molecular-weight material in the urine.

DISCUSSION

A disubstituted ^{125}I-NCS was shown to have a distribution and excretion similar to those reported by others using higher doses of native drug and assaying by microbiological methods (21). The data were also similar to those obtained by Maeda *et al.* (11, 12) with tritiated bisuccinylneocarzinostatin. A novel finding in the mouse, however, was the presence of the drug in bile. In order to carry out detailed pharmacokinetic analysis, we selected a larger animal, the rat, for further studies.

The pharmacokinetics of radioiodinated NCS in healthy animals was consistent with a 3-compartment open pharmacokinetic model. The concentration of NCS at zero time corresponded to distribution of drug in a central compartment with a volume comparable to the extracellular fluid volume. The

Chart 3. Mean cumulative biliary excretion of ^{125}I-NCS in intact (*n* = 4) and anephric (*n* = 3) rats during 10 hr following single i.v. dose. *Bars,* S.E.

initial rapid decay of the blood NCS concentration was associated with redistribution of drug from the central compartment to a much larger peripheral compartment. However, elimination from the central compartment was relatively rapid, giving little predisposition to drug accumulation in tissues. The model predicted that 56% of the drug was in the peripheral compartment (both "shallow" and "deep") at 15 min, but only 30 and 15% were there by 3 and 8 hr, respectively.

The highest tissue concentration of NCS was seen in the kidney; the next highest was in the skin, followed by the lungs, intestine, pancreas, and liver. The brain contained little if any drug, indicating limited passage of NCS across the blood-brain barrier. In the mouse and the rat, the testes contained the next lowest concentration of drug. Both the brain and the testes represent sites of relapse in acute leukemia in humans (3), in which NCS has been shown to have some activity (8, 14).

Renal excretion of intact NCS accounted for about 72% of total clearance. The renal clearance estimate (7.3 ml/min/kg) was less than inulin clearance in a normal 250-g rat, suggesting that NCS undergoes tubular reabsorption. However, this does not explain the discrepancy between plasma and renal elimination half-lives, which in theory should be identical.

Only a small percentage of the administered dose of NCS was excreted in the bile in either the mouse or the rat. Although bile excretion increased in the anephric rat, it proceeded at a maximum rate of about 0.3%/hr. This would account for only about 7% of the dose in 24 hr and could not compensate for absent renal excretion.

NCS exreted in the urine was chromatographically unchanged from native drug by gel filtration and was still biologically active. The results are consistent with the proposed mechanism of action whereby cytotoxicity may involve drug interaction with the cell membrane rather than extensive cellular uptake and metabolism (10).

The i.m. Walker tumor against which NCS has been shown to be active at a dose roughly equivalent to the dose administered in these studies showed a pattern of uptake and elimination similar to other rat tissues. However, radioactivity persisted in Walker tumor ascites, although not in L1210 ascites,
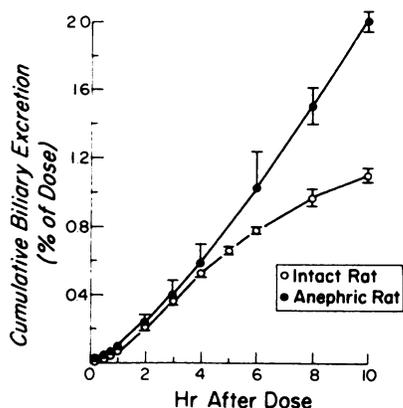

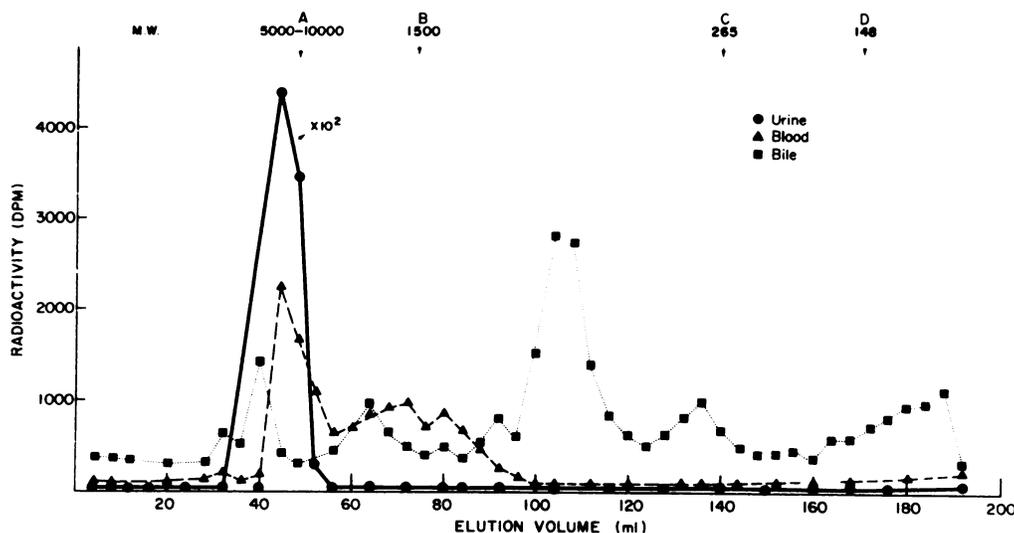Chart 4. Sephadex G-15 column (1.5 x 90 cm) chromatography of pooled (*n* = 4) plasma, urine, and bile specimens 1 hr after single i.v. injection of ^{125}I-NCS. Elution positions: *A,* NCS, insulin, and adrenocorticotropic hormone; and *B,* actinomycin D; *C,* 3-(4-hydroxyphenyl)propionic acid glycine conjugate; *D,* sodium iodide.

and suggested the possibility in some instances of "reservoir kinetics" which could potentially lead to delayed clearance of drug. Since in the present studies there were rapid elimination of NCS from the blood and little persistence of the drug in the tissues, optimum scheduling, at least in tumor model systems, might be frequent single injections or short continuous infusions.

It is believed that ^{123}I-NCS or ^{131}I-NCS could be used for studies of drug distribution in human patients, perhaps using external detectors and imaging devices as has been done with other labeled chemotherapeutic agents (7).

ACKNOWLEDGMENTS

REFERENCES

1. Bradner, W. T., and Hutchison, D. J. Neocarzinostatin (NCS-69856): an antitumor antibiotic effective against ascitic leukemic L1210 in mice. Cancer Chemother. Rep., 50: 79–84, 1966.
2. Bolton, A. E., and Hunter, W. M. The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. Biochem. J., 133: 529–539, 1973.
3. Eden, O. B. Testicular disease in acute lymphoblastic leukemia in childhood. Report on behalf of the Medical Research Council's working party in leukemia in childhood. Br. Med. J., 1: 334–338, 1978.
4. Gibaldi, M., and Perrier, D. Pharmacokinetics. New York: Marcel Dekker, Inc., 1975.
5. Greenblatt, D. J., and Koch-Weser, J. Clinical pharmacokinetics. N. Engl. J. Med., 293: 702–705, 1975.
6. Griffin, T., Comis, R. L., Lokich, J., and Cannellos, G. Neocarzinostatin: Phase I Study. Proc. Am. Assoc. Cancer Res., 18: 302, 1977.
7. Grove, R. B., Eckelman, W. C., and Reba, R. C. Preparation, distribution, and tumor imaging properties of ^{111}In, ^{57}Co, ^{67}Ga and ^{59}Fe labeled bleomycin. J. Nucl. Med., 14: 627, 1973.
8. Hiraki, K., Kamimua, D., Takahashi, I., Nagao, T., Kitajima, K., and Irino, S.

Neocarzinostatin, a new chemotherapeutic approach to acute leukemia. Nouv. Rev. Fr. Hematol., 13: 445–451, 1973.
9. Ishida, N., Miyazaki, K., Kumagai, K., and Rikimaru, M. Neocarzinostatin, an antitumor antibiotic of high molecular weight. Isolation, physicochemical properties and biological activities. J. Antibiot. (Tokyo) Ser. A, 18: 68–76, 1965.
10. Lazarus, H., Raso, V., and Samy, T. S. A. In vitro inhibition of human leukemic cells (CCRF-CEM) by agarose-immobilized neocarzinostatin. Cancer Res., 37: 3731–3736, 1977.
11. Maeda, H., Sakamoto, S., and Ogata, J. Mechanism of accumulation of the antitumor protein antibiotic neocarzinostatin in bladder tissue: intravenous administration, urinary excretion, and absorption into bladder tissue. Antimicrob. Agents Chemother., 11: 941–945, 1977.
12. Maeda, H., Yamamoto, N., and Yamashita, A. Fate and distribution of ^{14}C succinyl-neocarzinostatin in rats. Eur. J. Cancer, 13: 856–870, 1976.
13. Marquardt, D. W. An alogorithm for least squares estimation of nonlinear parameters. SIAM J. Appl. Math., 11: 431–441, 1963.
14. Masaoka, T., Hasegawa, Y., Ueda, T., Takubo, T., Shibata, H., Nakamura, H., Tatsumi, N., Yoshitake, J., and Senda, N. On the mode of decrease of leukemic cells during treatment of acute leukemia. Eur. J. Cancer, 12: 143–149, 1976.
15. Riegelman, S., Loo, J. C. K., and Rowland, M. Shortcoming in pharmacokinetic analysis by conceiving the body to exhibit properties of a single compartment. J. Pharm. Sci., 57: 117–123, 1968.
16. Samy, T. S. A. Neocarzinostatin: Effect of modification of side chain amino and carboxyl groups on chemical and biological properties. Biochemistry, 16: 5573–5578, 1977.
17. Samy, T. S. A., Hu, J-M., Meienhofer, J., Lazarus, H., and Johnson, R. K. A facile method of purification of neocarzinostatin. J. Natl. Cancer Inst., 58: 1765–1768, 1977.
18. Samy, T. S. A., Modest, E. J., and Johnson, R. K. Effect of modification of amino and carboxyl groups on the antitumor properties of neocarzinostatin. Proc. Am. Assoc. Cancer Res., 18: 124, 1977.
19. Samy, T. S. A., and Raso, V. Radioimmunoassay of neocarzinostatin, an antitumor protein. Cancer Res., 36: 4378–4381, 1976.
20. Takahashi, M., Toshiyama, K., Maeda, H., Kikuchi, M., Kumagai, K., and Ishida, N. Clinical trials of a new antitumor polypeptide neocarzinostatin (NCS). Tohoku J. Exp. Med., 98: 273–380, 1969.
21. Toriyama, K., Fujita, H., and Ishida, N. Absorption, distribution and excretion of neocarzinostatin (NCS) in mice after oral administration. J. Antibiot. (Tokyo) Ser. A, 28: 64–72, 1975.
22. Usanis, R. A. NLIN-Nonlinear Least Squares Estimation of Parameters. Library Services Series Document No. LSR-089-1. Research Triangle Park, N. C.: Triangle Universities Computation Center, 1972.
23. Wagner, J. G. Fundamentals of Clinical Pharmacokinetics. Hamilton, Ill.: Drug Intelligence Publications, 1975.