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## Absence of anti-neocarzinostatin (NCS) antibody production in leukemia patients treated with NCS

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# Absence of anti-neocarzinostatin (NCS) antibody production in leukemia patients treated with NCS\*

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## Abstract

Neocarzinostatin (NCS) was first used by Hiraki and his colleagues for induction chemotherapy in acute leukemia. This new anti-tumor agent is a polypeptide with a high molecular weight of 10,700 daltons. Anti-NCS antibody was produced in rabbits administered NCS intramuscularly with or without adjuvant. The production of anti-NCS antibody in patients treated with NCS was investigated. Forty three leukemia cases of various types were examined totally 65 times. Two mg of NCS for four consecutive days by intravenous drip infusion followed by 7 to 10 days of pause was repeatedly administered. The total amounts ranged 8 to 174 mg and the total periods 4 to 87 days. The methods used to measure the antibody titer are the passive hemagglutination (PHA) test on microplate and the passive cutaneous anaphylaxis (PCA) reaction in guinea pigs. The sera of all patients showed only non-specific agglutination at less than 2(3) dilution by PHA test, and to confirm these results four patient sera were tested by PCA reaction. The production of anti-NCS antibody was not detected in patients by PHA test and PCA reaction. The anaphylactic reaction and other adverse reactions due to anti-NCS antibody production were not demonstrated in patients. Anti-NCS antibody was not detected by these experiments in the dose schedule administered.

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**ABSENCE OF ANTI-NEOCARZINOSTATIN (NCS)  
ANTIBODY PRODUCTION IN LEUKEMIA  
PATIENTS TREATED WITH NCS**

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*Abstract.* Neocarzinostatin (NCS) was first used by Hiraki and his colleagues for induction chemotherapy in acute leukemia. This new anti-tumor agent is a polypeptide with a high molecular weight of 10,700 daltons. Anti-NCS antibody was produced in rabbits administered NCS intramuscularly with or without adjuvant. The production of anti-NCS antibody in patients treated with NCS was investigated. Forty three leukemia cases of various types were examined totally 65 times. Two mg of NCS for four consecutive days by intravenous drip infusion followed by 7 to 10 days of pause was repeatedly administered. The total amounts ranged 8 to 174mg and the total periods 4 to 87 days. The methods used to measure the antibody titer are the passive hemagglutination (PHA) test on microplate and the passive cutaneous anaphylaxis (PCA) reaction in guinea pigs. The sera of all patients showed only non-specific agglutination at less than 2<sup>3</sup> dilution by PHA test, and to confirm these results four patient sera were tested by PCA reaction. The production of anti-NCS antibody was not detected in patients by PHA test and PCA reaction. The anaphylactic reaction and other adverse reactions due to anti-NCS antibody production were not demonstrated in patients. Anti-NCS antibody was not detected by these experiments in the dose schedule administered.

Neocarzinostatin (NCS) is an anti-tumor antibiotic, which was first isolated from culture filtrate of *Streptomyces carzinostaticus* by Ishida *et al.* in 1956 (1). This agent has been chemically proved to be an acidic single-chained polypeptide with molecular weight of 10,700, consisted of 109 L-amino acids of 18 kinds (2).

Since 1972, NCS has been applied for the first time by Hiraki *et al.* (3) for the induction therapy of human acute leukemia, and its anti-leukemic effect has been evaluated and proved to be excellent (4).

Because NCS has enough large size of molecular weight to produce the antibody, the antigenicity of NCS has been demonstrated by Mukojima *et al.* (5) and by Tsujino *et al.* (6) in their animal experiments.

Investigation of the anti-NCS antibody in leukemia patients administered with NCS is considered to be important in preventing the adverse reactions of

NCS and in anticipating the loss of therapeutic effectiveness of this agent. Therefore, first, the anti-NCS antibody was produced in rabbits. Then, the leukemia patients' sera were investigated by the passive hemagglutination (PHA) test to search the anti-NCS antibody, and the questionable sera were confirmed by the passive cutaneous anaphylaxis (PCA) reaction in guinea pigs to rule out non-specific reactions.

## MATERIALS AND METHODS

(1) *Method of the anti-NCS antibody production in rabbits.*

Rabbits, weighing 2.4-3.1 kg, were divided into 3 groups, consisted of 2 in each.

NCS, 0.4-0.6mg (as shown in Table 1) was thoroughly homogenized with

TABLE 1 METHOD OF THE ANTI-NCS ANTIBODY PRODUCTION IN RABBITS

Group I (#1, #2) NCS (mg) + Freund's complete adjuvant; I. M.	↓	2w	↓	2w	↓	2w	↓	1w	↓	1w	↓	serum
	0.4 mg		0.5 mg		0.6 mg		0.5 mg		0.5 mg			
Group II (#3, #4) NCS (0.1 mg) + Normal Saline I. M.	4 days ↓↓↓	10d	↓↓↓	10d	↓↓↓	10d	↓↓↓	3d	↓↓↓	3d	↓↓↓	serum
Group III (#5, #6) NCS (0.1mg) I. V.	4 days ↓↓↓	10d	↓↓↓	10d	↓↓↓	10d	↓↓↓	3d	↓↓↓	3d	↓↓↓	serum

the same amount of Freund's complete adjuvant (Difco Laboratories), and this mixture was intramuscularly injected for sensitization in group I. The sensitizing procedures were done 5 times in every 2 weeks, and the sera sample was drawn one week after the final sensitization.

Groups II and III were sensitized according to the schedule modified from the protocol of human leukemia treatment for dose and administration method of NCS.

To group II, NCS 0.1 mg (0.04 mg/kg/day) without any adjuvant for 4 consecutive days was given intramuscularly and repeatedly, and to group III the same dose was given intravenously as shown in Table 1.

NCS used experimentally and clinically in this study was supplied by Kayaku Antibiotics Research Company. (Tokyo, Japan)

(2) *Leukemia patients treated with NCS.*

The investigated sera were drawn from 43 leukemia cases of various types on several occasions after the repeated NCS administration. Cases were 19 acute myelogenous leukemia, 4 acute promyelocytic leukemia, 2 monocytic leukemia, 1 erythroleukemia, 11 acute lymphocytic leukemia and 6 chronic myelogenous leukemia in blast crisis.

The method of administration of NCS, alone or in combination with conventional antileukemic agents, was followed by the protocol; 2mg of NCS daily for 4 consecutive days by intravenous drip infusion followed by 7-10 days of pause. The same course trials have to be repeated until the remission criteria will be satisfied.

The controls were sera of 14 healthy subjects and of 5 leukemia patients without NCS administration.

(3) *Passive hemagglutination (PHA) test by microplate method.*

Anti-NCS antibody was examined by the passive hemagglutination test and the hemagglutination inhibition test using the microplate method according to Mukojima *et al.* (5). The NCS binding sheep red blood cell and the PHA medium used in this experiment were the products of Nippon Kotai Kogyo K.K.

(4) *Passive cutaneous anaphylaxis (PCA) reaction in guinea pigs.*

Guinea pigs, weighing about 300-400g, of Hartley line, were divided into 6 groups, consisted of 3 in each.

The investigated sera were intradermally injected in the skin of shaved dorsum; 0.1ml of sera and adequately diluted sera with normal saline in several grades were prepared.

Four hr after the above pre-treatment, the mixture of NCS (1mg) and normal saline (0.5ml) tinged with 1% Evans blue (totally 1.5ml) was injected intravenously. Exactly 30min later, guinea pigs were sacrificed by head blow and the dorsum was immediately skinned. The diameters of the pigmented plaques at injected sites beneath the dorsal skin were measured.

## RESULTS

(1) *Anti-NCS antibody in rabbits by PHA test.*

As shown in Table 2, in group I, the serum from No. 1 rabbit showed the hemagglutination titer of  $2^5$  and No. 2 rabbit achieved the titer up to  $2^9$ , which was sensitized with NCS mixed with Freund's complete adjuvant.

In group II, which received only NCS intramuscularly, No. 4 rabbit showed

TABLE 2 HEMAGGLUTINATION TEST OF THE ANTI-NCS ANTIBODY IN RABBITS

Group	Rabbit	Dilution ( $2^n$ )										
		$2^0$	$2^1$	$2^2$	$2^3$	$2^4$	$2^5$	$2^6$	$2^7$	$2^8$	$2^9$	$2^{10}$
I	#1	+	+	+	+	+	+	-	-	-	-	-
	#2	+	+	+	+	+	+	+	+	+	+	-
II	#3	+	+	-	-	-	-	-	-	-	-	-
	#4	+	+	+	+	+	-	-	-	-	-	-
III	#5	±	-	-	-	-	-	-	-	-	-	-
	#6	±	-	-	-	-	-	-	-	-	-	-

the titer of  $2^4$ . But in group III, which received only NCS intravenously, the sera showed no elevated titer of hemagglutination.

By the method of the hemagglutination inhibition test, in which the investigated sera were incubated with NCS for one hr to inactivate the effect of antibody, the hemagglutinations were reversely inhibited, as shown in Table 3. And these results indicated that the above hemagglutinations were specific to the presence of the anti-NCS antibody.

TABLE 3 HEMAGGLUTINATION INHIBITION TEST OF THE ANTI-NCS ANTIBODY IN RABBITS

Rabbit		Dilution ( $2^n$ )										
		$2^0$	$2^1$	$2^2$	$2^3$	$2^4$	$2^5$	$2^6$	$2^7$	$2^8$	$2^9$	$2^{10}$
#2	control	+	+	+	+	+	+	+	+	+	+	-
	inhibition	+	+	-	-	-	-	-	-	-	-	-
#4	control	+	+	+	+	+	-	-	-	-	-	-
	inhibition	-	-	-	-	-	-	-	-	-	-	-
#6	control	-	-	-	-	-	-	-	-	-	-	-
	inhibition	-	-	-	-	-	-	-	-	-	-	-

(2) *The investigation in leukemia patients treated with NCS, by PHA test screening.*

Forty three leukemia cases were examined totally 65 times. The total amounts of NCS administered ranged 8 to 174 mg, and the total periods of NCS administration ranged 4 to 87 days.

All of the investigated sera showed only the non-specific agglutination in less than  $2^3$  dilution, and the questionable sera were confirmed by the hemagglutination inhibition test. Two cases, who showed the titer of  $2^3$  dilution, were the patients S.T. and C.M., and their sera were re-examined by the passive cutaneous anaphylaxis reaction in guinea pigs, in the following experiment.

(3) *Anti-NCS antibody detection in sensitized rabbits and in leukemia patients by PCA.*

The results were shown in Table 4 and in Figures 1 and 2.

In group I, the established anti-NCS anti-serum of No. 2 rabbit gave the positive titer of 1 : 1000 dilution, and in group II, the result showed the titer of 1 : 100 dilution by the serum of No. 4 rabbit.

In 4 groups (III to VI), patients' sera were investigated to rule out the presence of anti-NCS antibody. In groups IV and V, 2 patients (S.T. and C.M.) were chosen because their sera had the titer of  $2^3$  dilution by PHA test. In groups III and VI, other two patients (K.U. and N.I.) were chosen because they developed shock possibly due to the injection of NCS. K.U.'s serum was drawn

TABLE 4 PASSIVE CUTANEOUS ANAPHYLAXIS (PCA) REACTION OF THE ANTI-NCS ANTIBODY IN GUINEA PIGS

Group	Serum Dilution					Normal Saline	Control Normal Serum	Rabbit #2 Serum (1:1)
	1:1	1:10	1:100	1:500	1:1000			
I. Anti-NCS serum of #2 rabbit	###	##	++	++	+	-	(i)	-
II. Anti-NCS serum of #4 rabbit	##	++	+	-	-	-	(ii)	-
III. Pt's serum (K.U.) (S48. 12. 30)	-	-	-	-	-	-	(iii)	##
IV. Pt's serum (S.T.) (S50. 5. 24)	-	-	-	-	-	-	(iii)	##
V. Pt's serum (C.M.) (S49. 10. 7)	-	-	-	-	-	-	(iii)	##
VI. Pt's serum (N.I.) (S50. 4. 12)	-	-	-	-	-	-	(iii)	##

Criteria: Diameters averaged in each group

(-) no reaction	(++) 10-15 mm	(i) #2 rabbit control serum
(±) <5 mm	(##) 15-20 mm	(ii) #4 rabbit control serum
(+) 5-10 mm	(###) >20 mm	(iii) normal human serum

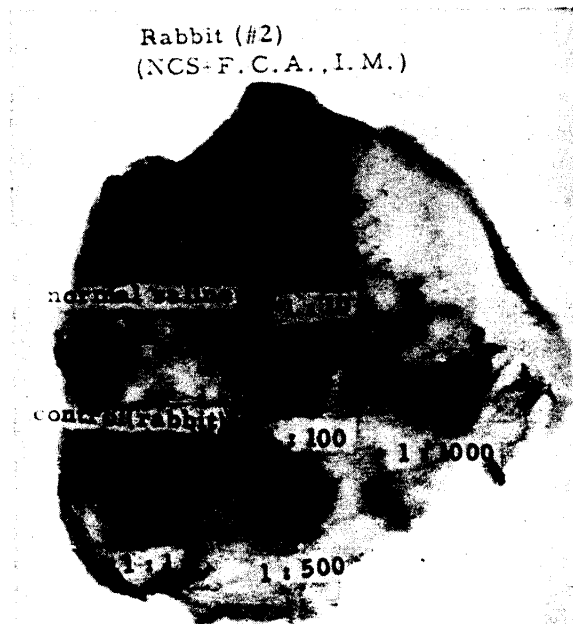


Fig. 1. PCA reaction of No. 2 rabbit. Positive reaction is seen up to the site of 1:1000 dilution.



Fig. 2. PCA reaction of pt. (K. U.). Negative reaction is seen even in the site of 1:1 dilution. Control of NCS antiserum is positive.

just on the day after the shock episode and N.I.'s serum was drawn on the next day after the shock.

As shown in Table 4, there showed no presence of anti-NCS antibody at all in the leukemia patients.

#### DISCUSSION

The antigenicity of NCS has been proved by this animal experiment and by the papers reported previously.

And clinically, investigation of anti-NCS antibody in the patients treated with enough amount of NCS has been carried out first time in this study.

L-asparaginase, which is an anti-tumor agent of high molecular protein (molecular weight 100,000-160,000) and is refined from *E. coli*, caused the antibody; this resulted in the loss of therapeutic effectiveness according to a murine experiment (7).

Investigation of anti-L-asparaginase antibody during L-asparaginase therapy in leukemia patients by the passive hemagglutination method showed the high titer (1:1000-1:5000 dilution) in 14.8% patients (8).

And in another paper (9), the allergic phenomenon, such as urticaria and



fever, in a patient treated with L-asparaginase was proved to be related with the IgG antibody production by PCA and radioimmuno-electrophoresis.

In this study, the anti-NCS antibody was produced easily in rabbits, when NCS was given intramuscularly without any adjuvant. But in case of the intravenous administration no antibody was detected.

This experimental result suggests that no antibody was detected at least by PHA method in human leukemia cases, to whom NCS was administered intravenously and repeatedly.

The PCA reaction has been done to confirm the results of the PHA test.

The serum of No. 2 rabbit had the titer of  $2^9$  (=1:512 dilution) by PHA and was related with the titer of 1:1000 dilution by PCA. Similarly the serum of No. 4 rabbit had the titer of  $2^4$  (=1:16 dilution) by PHA and the titer of 1:100 dilution by PCA. A slight dissociation between the titer of PHA and that of PCA is considered to be due to more sensitive PCA than PHA for the detection of a very small quantity of IgG antibody.

The negative data of two cases (K.U. and N.I.) are important to rule out the possibility of anaphylactic reaction due to NCS antibody clinically. As these two patients have never been in shock again up to now with other various medications, still NCS is thought to be a causative agent for shock, although the shock episodes in these two cases were successfully treated with medical procedures. These data can rule out the interference of NCS antibody for the possible anaphylaxis and other adverse reactions in leukemia patients.

The reasons of no detectable antibody production by intravenous administration of NCS can be discussed as follows. (i) NCS is rapidly excreted in urine and the concentration level in blood is decreased, when NCS is given by intravenous route (10). (ii) NCS has the immunosuppressive effect on the antibody production (11). (iii) Most of the conventional antileukemic agents in combination with NCS, when treating the human leukemia, have the immunosuppressive effects as well. (iv) The immuno-surveillance system is considered to be generally depressed in leukemia patients, especially of the acute phase.

Although the production of antibody against the antileukemic antibiotic is the interesting phenomenon in experimental work, the clinical analysis of antibody detection should be investigated by further studies. The anaphylactic reactions in human, which is proved to be due to the antibody production against NCS, has not been found yet, and the hypersensitivity against NCS is considered to be only a factor causing this rare, undesirable reaction.

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