

Original research

Effects of glycyrrhizin (SNMC: Stronger Neo-Minophagen C®) in hemophilia patients with HIV-1 infection

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Forty-two hemophiliacs with HIV infection were treated with high-dose glycyrrhizin, STRONGER NEO-MINOPHAGEN C® (SNMC). The dose was 100-200 ml of SNMC in 21 patients and 400-800ml in the other 21. The patients were divided into an asymptomatic carrier (AC) group and an AIDS-related complex (ARC)/AIDS group. The SNMC was administered intravenously daily for the first 3 weeks, and every second day for the following 8 weeks to the 42 HIV-infected hemophilia patients, in accordance with the protocol proposed by the Japanese National Research Committee. The CD4/CD8 ratio and the CD4 positive lymphocyte counts did not change during the treatment period. However, significant improvement was noted in some cases. A slight increase in mitogenic responsiveness to phytohemagglutinin, Concanavalin A and pokeweed mitogen was noted in most patients in both groups, significant improvement was seen in the AC group administered over 400ml of SNMC. Furthermore, complete recovery was noted in liver dysfunction, which has been thought to be one of the major problems with hemophiliacs treated with blood products. Thus, prophylactic administration of high dose SNMC to HIV positive hemophiliacs who have impaired immunological ability and liver dysfunction was considered to be effective in preventing the development from AC/ARC to AIDS.

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GLYCYRRHIZIN (GL) IS AN AQUEOUS EXTRACT PRODUCT OF LICORICE ROOT *(Glycyrrhiza radix)*, which is known as an anti-inflammatory substance in Chinese medicine. This compound consists of one molecule of glycyrrhetinic acid and two molecules of glucuronic acid. Purified GL dissolved in saline together with glycine and cysteine, under the trade name of STRONGER NEO-MINOPHAGEN C (SNMC), has been administered intravenously to allergic and dermatological patients and has also been reported to have therapeutic and prophylactic effects in cases of chronic active viral hepatitis (Fujisawa et al. 1980). It is widely used in Japan. Furthermore, when given intravenously in small doses, GL has been demonstrated to exhibit interferon-inducing and NK-enhancing activity, not only in mice, but also in humans (Abe et al. 1984; Ito and Kumagai 1984). In addition, Ito et al. (1987) recently proved that GL has an anti-HIV-1 effect in vitro. Based on these obervations, we have attempted to use this drug in HIV-infected hemophilia patients, hoping to prevent the development of AC/ARC to AIDS (Yamada 1987).

	I (<200ml)	II (>400ml)	Total
AC	14	14	28
ARC	4 } 7	5 } 7	9 } 14
AIDS	3 }	2 }	5 }
Total	21	21	42

TABLE 1. Dosage of drug and number of cases

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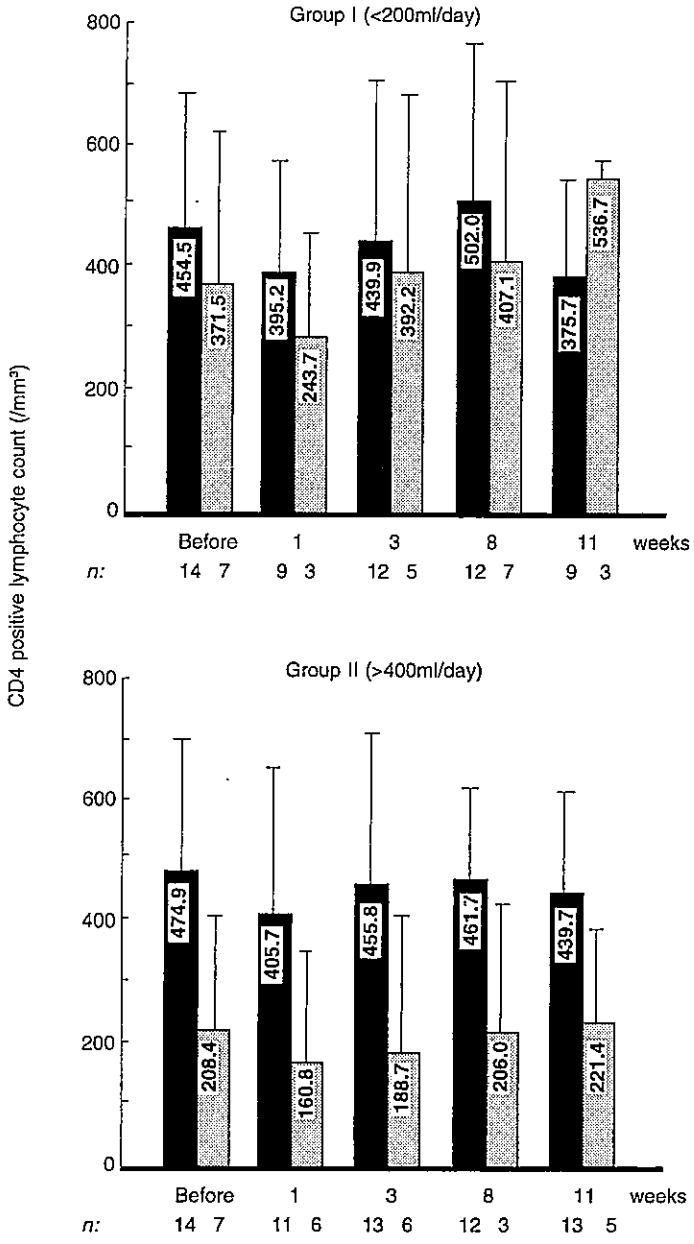

FIGURE 1. Changes in CD4 positive lymphocyte count after intravenous administration of SNMC. ■ AC; ▨ ARC/AIDS; *n*, number

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Materials and Methods

Forty-two hemophiliacs with HIV-1 infection, residing in the Tohoku district of Japan, were treated with high-dose SNMC *(Table 1)*. They were divided in to groups: Group I receiving less than 200ml daily and Group II taking an excess of 400ml daily.

SNMC was administered intravenously daily for the first 3 weeks, and every second day for the following 8 weeks, to 42 HIV-1 antibody positive hemophilia patients, in accordance with the protocol proposed by the Japanese National Research Committee.

CD4 positive lymphocyte count (flow cytometry), CD4/CD8 ratio (flow cytometry), and mitogenic responsiveness of lymphocytes to PHA (phytohemagglutinin), ConA (concanavalin A) and PWM (pokeweed mitogen) were examined before and at 1, 3, 8 and 11 weeks after the administration of SNMC. Other routine laboratory tests, such as liver function tests (GOT, GPT), renal function tests (BUN, S-Cr, UA), serum lipids (TC, Tg) etc. were also performed. These data were evaluated statistically at the above-mentioned five points and classified as either improvement (effective), no change, or deterioration.

Results

CD4 positive lymphocyte count transiently increased from 454.5/mm3 to 502/mm3 (max. value) at the end of the 8th week in AC group patients administered under 200ml of SNMC (group I). It also increased from 371.5mm³ to 536.7mm³ in ARC/AIDS group at the end of 11th week. However these changes were not statistically significant during the treatment period in all groups. Almost the same results were obtained in the group administered over 400ml (group II- *Figure 1)*.

CD4/CD8 ratio increased from 0.48 to 0.57 (max. value) at the end of 8th week in the AC patients of group I. A significantly lower value (0.25) was

noted in ARC/AIDS patients than AC patients, and CD4/CD8 ratio in group I patients showed only slight fluctuations and neither increased nor decreased significantly during the treatment period. Almost the same results were noted in group II patients *(Figure 2)*.

Mitogenic responsiveness of lymphocytes to PHA showed a definite increasing tendency from 32,470 cpm to 39,746 cpm at the end of 11th week in AC patients of group I, but these data were not statistically significant. However, 10 cases of AC patients of group II showed a significant improvement from 31,945 cpm to 40,297 cpm ($p<0.01$) at the end of the 3rd week and to 39,519 cpm ($p<0.05$) at the end of the 11th week. However, no improvement or deterioration was noted in 6 cases of ARC/AIDS patients *(Figure 3)*.

Almost the same results were obtained in mitogenic responsiveness of lymphocytes to ConA. AC patients of group I showed a nonsignificant but definite increasing tendency. AC patients of group II showed significant improvement ($p<0.05$) at the end of the 8th and 11th weeks *(Figure 4)*. In mitogenic responsiveness to PWM, significant improvement ($p<0.05$) was obtained, even in AC patients of group I *(Figure 5)*.

Changes in clinical symptoms and complications *(Table 2)*: Four cases of lymphadenopathy, 1 case of oral candidiasis and 1 case of hepatosplenomegaly noted in AC patients of group I, and 1 case of upper respiratory tract infection in AC patients of group II improved after the administration of SNMC. The disappearance of lymph node swellings in 3 cases, and oral candidiasis and aphthae in one case, and improvement of anorexia, malaise and fatigue in 2 cases and of night sweats in one case were noted in the ARC/AIDS cases of group I. More prominent improvement was recognized in 7 ARC/AIDS cases of group II; in particular, it should be noted that in one patient, posi-

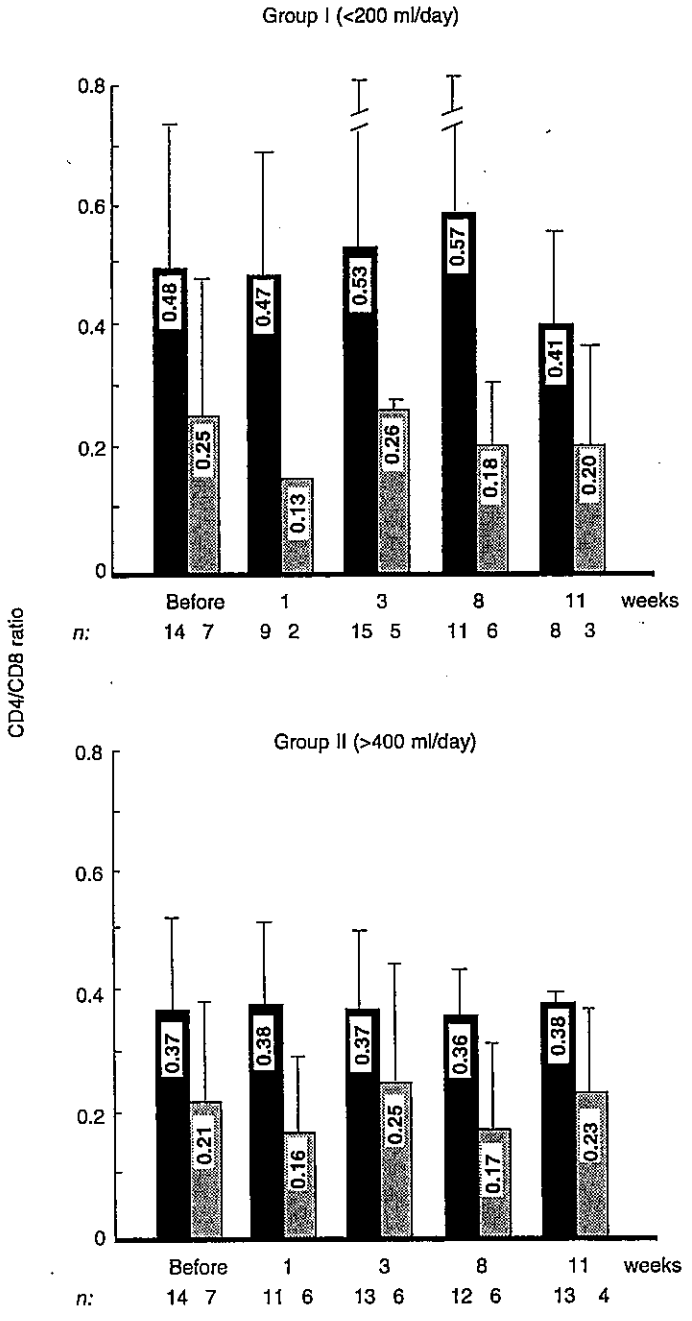

FIGURE 2. Changes in CD4/CD8 ratio after intravenous administration of SNMC. ■ AC; ▨ ARC/AIDS; *n*, number

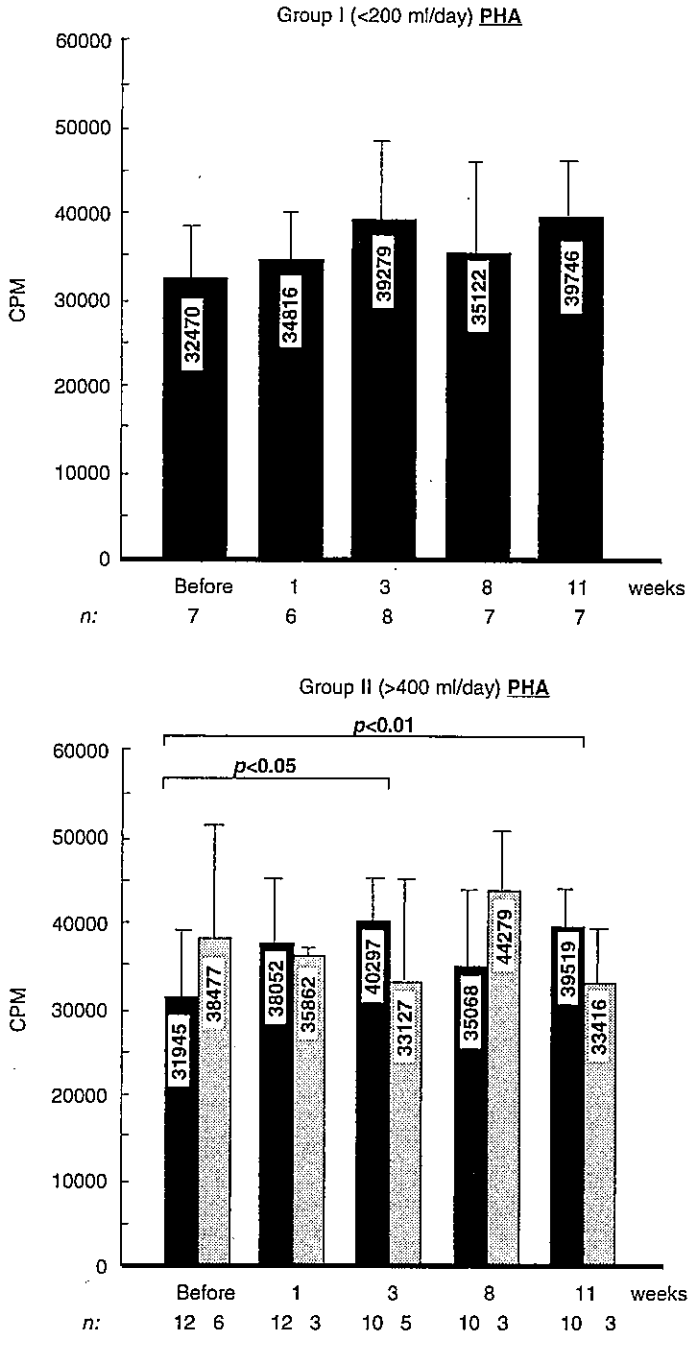

FIGURE 3. Changes in mitogenic responsiveness of lymphocytes to PHA after intravenous administration of SNMC. ■ AC; ▨ ARC/AIDS; *n*, number

tive HIV-1 antigen disappeared after the administration of super-high dose of SNMC (800ml; 1,600mg of glycyrrhizin).

One complication was a decreasing tendency of serum K^+ ion noted in 4 cases of AC patients and 1 case of ARC/AIDS of group II, which improved promptly following the cessation of the drug, or supplementation of K^+ ion containing saline solution. Other complications were skin rash in one case of group I, and headache in one case of group II. However, no serious complications were recognized.

Case Report

S.K. *23 year old male, hemophilia A* (Table 3). He discovered that he was positive for HIV-1 antibody and antigen in 1986, thereafter, he rarely visited our clinic because he resided far away.

Around June 1988, he complained of a productive cough and his CD4/CD8 ratio was extremely low, 0.02 and again positive antigen was noted. SNMC (800ml) was administered daily for the first 8 weeks and then the dose was decreased gradually. HIV-1 antigen disappeared shortly after the administration of SNMC, thereafter, he received 300mg/day of 3'-azide-2',3'-dideoxythymidine (AZT) orally. The negative state of HIV-1 antigen continued for about 10 months.

This change is similar to a case using a comparable high dose of SNMC reported by Hattori et al. (1989). This finding proves an anti-HIV effect of the drug in vivo, as already shown by Ito et al. (1987) in vitro, and argues for the usefulness of high-dose administration of SNMC in HIV-1 antigen positive patients. Recently, however, this case died following the onset of full-blown AIDS.

Discussion

1) CD4 positive lymphocyte count and CD4/CD8 ratio were not improved statistically. However, none of the cases showed a decreasing tendency in the ratios and predominant improvement was noted in some of these patients receiving high-dose of SNMC during the treatment course.

2) Mitogenic responsiveness of lymphocyte to PHA, ConA and PWM showed improvement in almost all cases, especially predominant in AC cases of group II (statistically significant).

3) Clinical symptoms, such as oral candidiasis and aphthae, lymph node swelling, skin rash, anorexia, malaise and hepatosplenomegaly, etc. improved or disappeared in several cases. Special attention should be paid to the one case in which p-24 antigen disappeared after the administration of 800ml of SNMC.

4) As already shown in our papers (Mori et al. 1989 a,b,c), liver dysfunction, noted in almost all hemophilia patients treated with blood products, was reversed in all cases. The grade of improvement was higher in group II than in group I.

The absence of changes in CD4 positive lymphocyte count and CD4/CD8 ratio argue for efficacy, because most HIV-infected hemophilia patients exhibit deterioration during their course. However, a longer period of study is required for the evaluation of the true efficacy of this drug and presently a trial study group is under way. Although our data consisted of the results of many departments of universities and hospitals in the Tohoku district and the time of sample collection and maximum points were slightly different in each case, statistically significant improvement was not obtained on average values. However, several cases showed marked improvement.

Changes in mitogenic responsiveness of lymphocytes to PHA, ConA and PWM were statistically significant, especially in AC cases of group II. These

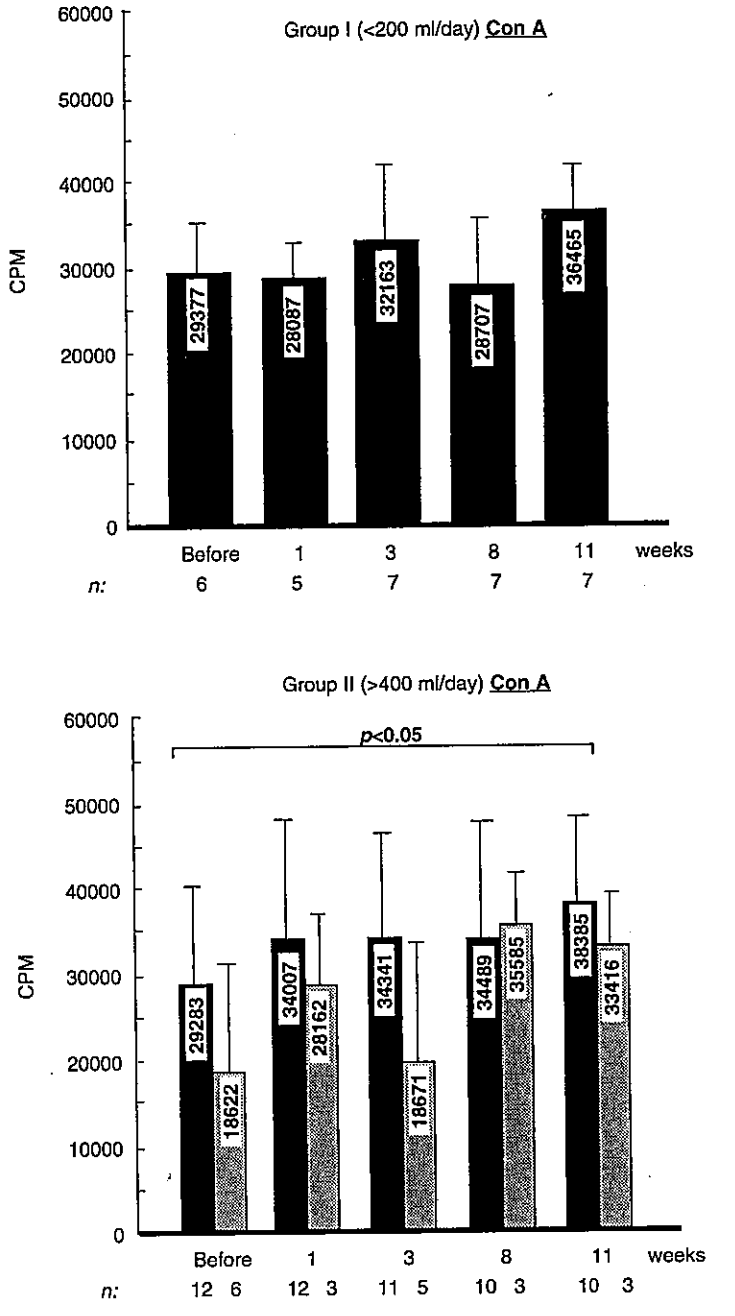

FIGURE 4. Changes in mitogenic responsiveness of lymphocytes to Con-A after intravenous administration of SNMC. ■ AC; ▒ ARC/AIDS; *n*, number

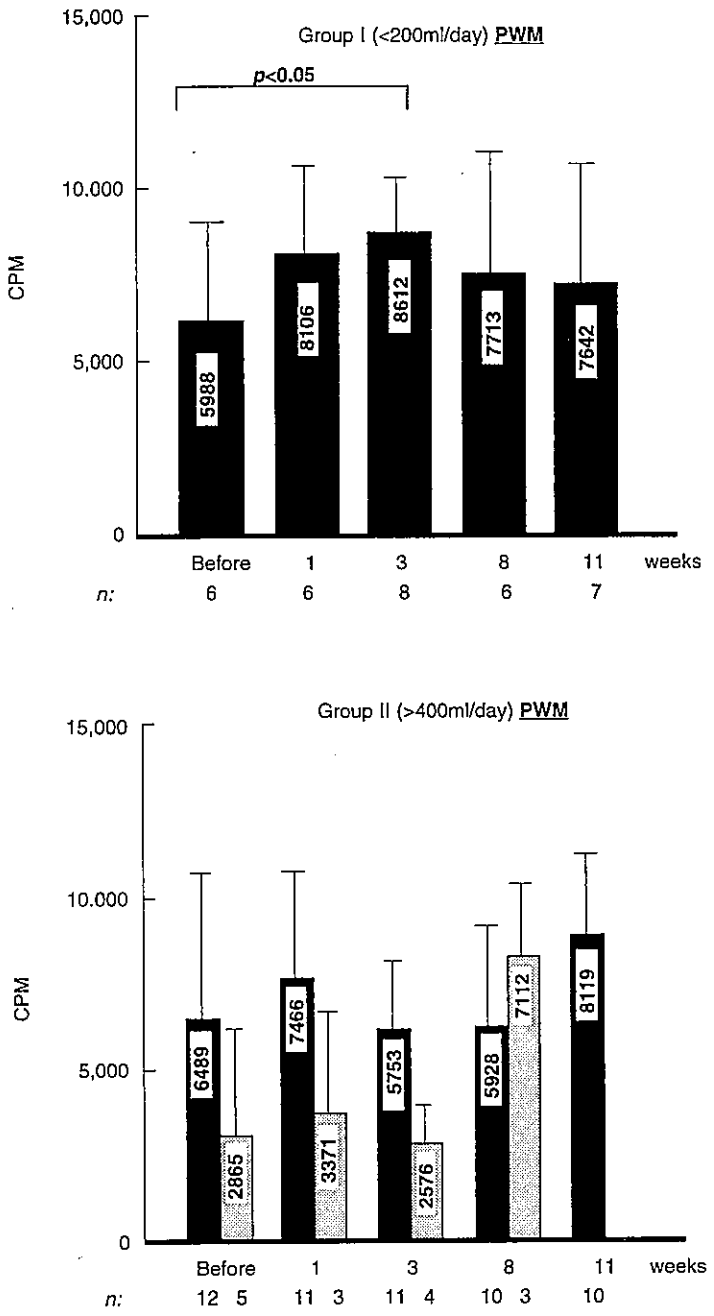

FIGURE 5. Changes in mitogenic responsiveness of lymphocytes to PWM after intravenous administration of SNMC. ■ AC; ▨ ARC/AIDS; *n*, number

results obtained under the above conditions are thought to be extremely valuable.

From the above-mentioned results, we now believe that these cases should be administered high-dose of SNMC initially in order to decrease the antigen under the cut-off level while p-24 antibody is maintained at an adequate value. Intermittent SNMC treatment may then in combination with other drugs, such as AZT, 2',3' dedeoxyinosine (ddI), intact g-globulin preparation, etc. prevent the development from AC/ARC to overt AIDS because of the maintenance of immunological function and suppression of virus growth.

Most hemophilia patients treated with blood products suffer from liver disease (chronic hepatitis) which is expected to progress to a more severe state. This is one of the major problems in hemophilia treatment. Administration of high-dose SNMC was more effective than the formerly recommended dose in improving liver dysfunction. In addition, SNMC is well-known to improve the adverse effects of anti-cancer drugs upon the hematopoetic, immune system and/or liver functions (Yamagata 1966; Matsunaga et al. 1968; Hoshino et al. 1972). From these facts, the combination of SNMC and other drugs such as, AZT, ddI and intact gamma-globulin preparations, etc. is expected to be useful in the treatment of hemophilia patients with HIV infection. We are going to try such a combination treatment program.

Conclusion

From the above results, administration of high dose SNMC is thought to be effective in the treatment of HIV-infected hemophilia patients based on the improvements observed in their clinical symptoms, immunological and liver functions, in particular after administration of a higher dosage of SNMC (group II).

	Improvement or disappearance			
	Group I		Group II	
AC 28 cases (14 cases each group)	Lymphadenopathy	4/4	Lymphadenopathy	0/2
	Oral candidiasis	1/11	Recurrent URI	1/1
	Hepatosplenomegaly	1/2		
	Night sweats	0/2		
	Malaise/ fatigue	0/1		
ARC/AIDS 14 cases (7 cases each group)	Lymphadenopathy	3/6	Lymphadenopathy	3/5
	Oral candidiasis	1/7	Oral candidiasis	2/2
	Anorexia	2/2	Anorexia	2/2
	Malaise/ fatigue	2/2	Malaise/ fatigue	2/2
	Oral apthae	1/1	p24 Ag	1/1
	Night sweats	1/1	Night sweats	2/2
	Exanthema	0/1	Exanthema	3/3
	Hepatosplenomegaly	0/1	Hepatosplenomegaly	1/1
	Complications			
	Erythema of arm and leg*	1	Hypokalemia+	5
			Headache/ vomiting*	1

* Required cessation of therapy + Improved by potassium supplement

TABLE 2. Changes in clinical symptoms and complications after administration of SNMC

			HIV:Ab	GP41:Ab		p24:Ab		HIV:Ag	
1986	August	11	(+)					(+)	
				[SNMC (800 ml/day administration]					
1988	March	14						(-)	
	March	22		(+)	101.	(-)	43.6		
	March	28						(-)	
	April	12						(-)	
	July	18		(+)	101.	(-)	24.3	(-)	
1989	January	19		(+)	100.	(-)	15.4	(-)	
	March	09		(+)	97.9	(-)	-6.0	(+)	0.127
	May	29						(+)	0.276
				[SNMC 600 ml/day, AZT 300 mg]					
	June	19						(+)	1.441
	August	03						(+)	>2.00

TABLE 3. Case report: S.K. 23 year-old male with hemophilia A

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