Effects of Glycyrrhizin (SNMC: Stronger Neo-Minophagen C®) in Hemophilia Patients with HIV Infection

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Glycyrrhizin (GL) not only has an inhibitory effect on HIV replication but also exhibits interferon-inducing and natural killer (NK)-enhancing effects and improves liver dysfunction. Thus, large doses of GL (200–800 mg/day) were intravenously administered for more than 8 weeks to 9 hemophilia A patients with HIV infection (asymptomatic carrier, AC). Lymphocyte count increased in all 9 cases. OKT4/OKT8 ratio was elevated in 6 out of the 9 cases and OKT4-positive lymphocytes increased in 8 out of the 9 cases; 66.7% and 88.9% improvement, respectively. Changes in NK cell activity and mitogenic responsiveness to PHA, Con A and PWM were not significant. Liver dysfunction, noted in 4 cases, clearly improved. Serum electrolytes, protein, lipids, and renal function were within normal levels and no serious side-effects were observed during treatment. On the other hand, in 3 cases of hemophilia without HIV infection, the number of OKT4 lymphocytes was not significantly altered during treatment. From these results, large dose administration of GL to HIV-positive hemophilia patients (AC) seems to be effective in preventing development of AC into AIDS by raising the number of decreased OKT4 lymphocytes and improving liver dysfunction.

Many compounds have been evaluated for their inhibitory effects on human immunodeficiency virus (HIV) replication in vitro (De Clercq 1986). Clinical trials have elucidated the potential of some of these drugs such as azidothymidine (Fischl et al. 1987). Serious side effects, however, have also been reported.

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Glycyrrhizin (GL) is an aqueous extract 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 glucoronic acid. GL in combination with glycine and cysteine (Stronger Neo-Minophagen C®, SNMC) has been reported to have therapeutic and prophylactic effects on chronic active viral hepatitis (Fujisawa et al. 1980), and widely used in Japan in the treatment of chronic viral hepatitis. 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 effect in vitro. Based on these observations, we attempted to use this drug in AC patients, hoping to prevent development of AC into AIDS (Yamada 1987).

**MATERIALS AND METHODS**

Nine cases of hemophilia A with HIV antibody (asymptomatic carrier, AC) and 3 cases of hemophilia A without HIV antibody as a control were examined before and 1, 3, 8 and 11 weeks after the administration of Stronger Neo-Minophagen C® (SNMC) consisting of 0.2% GL dissolved in saline and supplemented with 2% glycine and 0.1% cysteine (kindly provided by Minophagen Pharmaceutical Co., Tokyo).

Large doses of SNMC (100 ml in one case, 200 ml in 7 cases and 400 ml in one case per day) were administered intravenously daily for the first 3 weeks, every second day for the following 5 weeks and then twice a week until the end of the 11th week, to HIV AC patients.

**Table 1. Effect of SNMC administration**

<table>
<thead>
<tr>
<th>Patient</th>
<th>SNMC</th>
<th>Note</th>
<th>WBC Before</th>
<th>WBC After</th>
<th>LY. count Before</th>
<th>LY. count After</th>
<th>OKT4/8 ratio Before 3 weeks</th>
<th>OKT4/8 ratio Before 8 weeks</th>
<th>OKT4/8 ratio Before 11 weeks</th>
<th>OKT4/8 ratio Before 11 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV(−)1</td>
<td>100 ml</td>
<td></td>
<td>3600</td>
<td>3400</td>
<td>1200</td>
<td>1300</td>
<td>0.81</td>
<td>0.77</td>
<td>0.66</td>
<td>0.88</td>
</tr>
<tr>
<td>HIV(−)2</td>
<td>100 ml</td>
<td></td>
<td>4000</td>
<td>3600</td>
<td>800</td>
<td>1000</td>
<td>1.12</td>
<td>0.78</td>
<td>0.65</td>
<td>0.60</td>
</tr>
<tr>
<td>HIV(−)3</td>
<td>100 ml</td>
<td></td>
<td>5900</td>
<td>6000</td>
<td>2400</td>
<td>2700</td>
<td>0.74</td>
<td>0.71</td>
<td>0.63</td>
<td>0.55</td>
</tr>
<tr>
<td>III-3</td>
<td>100 ml</td>
<td></td>
<td>9600</td>
<td>9100</td>
<td>4900</td>
<td>6000</td>
<td>0.47</td>
<td>0.35</td>
<td>0.35</td>
<td>0.46</td>
</tr>
<tr>
<td>III-1</td>
<td>200 ml</td>
<td></td>
<td>5300</td>
<td>5100</td>
<td>1700</td>
<td>2300</td>
<td>0.30</td>
<td>0.38</td>
<td>0.52</td>
<td>0.38</td>
</tr>
<tr>
<td>III-2</td>
<td>200 ml</td>
<td></td>
<td>8700</td>
<td>5900</td>
<td>1800</td>
<td>2000</td>
<td>0.33</td>
<td>0.34</td>
<td>0.43</td>
<td>0.25</td>
</tr>
<tr>
<td>III-4</td>
<td>200 ml</td>
<td></td>
<td>8200</td>
<td>8600</td>
<td>1100</td>
<td>2600</td>
<td>0.57</td>
<td>0.59</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>III-5</td>
<td>200 ml</td>
<td></td>
<td>7100</td>
<td>8000</td>
<td>2100</td>
<td>5000</td>
<td>0.17</td>
<td>0.20</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>III-6</td>
<td>200 ml</td>
<td></td>
<td>2900</td>
<td>2400</td>
<td>2900</td>
<td>3500</td>
<td>0.57</td>
<td>0.55</td>
<td>0.79</td>
<td>1.36</td>
</tr>
<tr>
<td>III-7</td>
<td>200 ml</td>
<td>OKT4 deficient, leu3α(+)</td>
<td>6000</td>
<td>5700</td>
<td>2300</td>
<td>3200</td>
<td>0.46</td>
<td>0.34</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>III-9</td>
<td>200 ml</td>
<td></td>
<td>4300</td>
<td>3600</td>
<td>1000</td>
<td>1100</td>
<td>0.28</td>
<td>0.22</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>III-8</td>
<td>400 ml</td>
<td></td>
<td>5100</td>
<td>6600</td>
<td>2300</td>
<td>2500</td>
<td>0.37</td>
<td>0.80</td>
<td>0.59</td>
<td>0.51</td>
</tr>
</tbody>
</table>
with hemophilia A. Three cases of hemophilia A without HIV infection were also intravenously administered 100 ml of SNMC daily according to the above schedule.

The following laboratory tests and measurements were performed. Skin test (MULTITEST CMI®, manufactured by Institut Menieux, Lyon, France) (Kniker et al. 1979), OKT4/OKT8 ratio (flow cytometry), OKT4-positive lymphocyte count (flow cytometry), NK cell activity (¹¹¹Cr release assay), mitogenic responsiveness of lymphocyte to PHA (phytohemagglutinin), Con A (concanavalin A) and PWM ( pokeweed mitogen) (³H-thymidine uptake assay), liver function tests (GOT, GPT), renal function tests (BUN, blood urea nitrogen; Cr, serum creatinin; UA, uric acid), serum electrolytes (Na, sodium; K, potassium) and serum lipids (TC, total cholesterol; TG, triglyceride).

Data were evaluated according to the maximum value of increases or decreases of each parameter and were classified as either improvement, no change or deterioration.

**RESULTS**

Lymphocyte count increased in all AC patients, but no relationship between this increase and the dose of SNMC administered could be statistically established (Table 1). OKT4/OKT8 ratio increased in 6 cases (66.7% improvement) and the number of OKT4-positive lymphocytes also increased in 8 cases (88.9% improvement), the one exception being a case in the 200 ml group that was unchanged. There was one case in the 400 ml group, in which OKT4-positive lymphocytes increased predominantly from 513/mm³ before treatment to 1141/mm³ 3 weeks after treatment, which is within the normal range. Platelet count was normal in all cases and unchanged after the treatment. Liver dysfunction in 4 cases clearly improved after SNMC administration, with GOT and GPT in particular becoming asymptomatic carrier of hemophilia

<table>
<thead>
<tr>
<th>OKT4 lymphocyte count</th>
<th>Liver function</th>
<th>Platelet count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 3 weeks weeks 11 weeks weeks</td>
<td>GOT Before After Before After</td>
<td>GPT Before 1 week 3 weeks weeks 8 weeks weeks</td>
</tr>
<tr>
<td>389 473 355 387</td>
<td>58 15 74 42 19.0</td>
<td>16.9 23.1 21.3</td>
</tr>
<tr>
<td>332 361 322 290</td>
<td>137 91 176 127 17.2</td>
<td>17.8 16.9 18.2</td>
</tr>
<tr>
<td>758 845 707 724</td>
<td>30 36 49 47 23.3</td>
<td>19.9 20.3 19.7</td>
</tr>
<tr>
<td>1112 932 1260</td>
<td>35 55 32 35 34.5</td>
<td>33.9 36.0</td>
</tr>
<tr>
<td>245 518 491 400</td>
<td>45 21 80 48 20.6</td>
<td>16.9 18.5 20.7</td>
</tr>
<tr>
<td>387 301 421 322</td>
<td>28 9 27 20 23.9</td>
<td>33.1 32.6 31.2 31.6</td>
</tr>
<tr>
<td>274 977 691</td>
<td>113 28 556 11 23.1 29.6 25.7 27.7</td>
<td></td>
</tr>
<tr>
<td>262 423 940</td>
<td>78 48 101 53 20.3 18.6 20.0 19.7</td>
<td></td>
</tr>
<tr>
<td>248 346 433</td>
<td>64 30 120 48 18.9</td>
<td>19.7 19.5 19.9</td>
</tr>
<tr>
<td>541 611 673</td>
<td>24 50 38 64 15.2</td>
<td>15.2 12.7 15.3</td>
</tr>
<tr>
<td>161 141 143</td>
<td>32 30 43 31 28.3</td>
<td>27.5 23.1 22.5</td>
</tr>
<tr>
<td>513 1141 735 771</td>
<td>18 18 20 15 18.2</td>
<td>18.4 20.6 19.3 17.8</td>
</tr>
</tbody>
</table>
normalized in all cases and TTT and ZTT improving in some cases. These results are clearly superior to results previously reported for liver diseases.

In all three cases of HIV negative control, the numbers of lymphocytes and OKT4-positive lymphocytes were not significantly altered. The OKT4/OKT8 ratio, however, was clearly decreased in 2 cases (1.12→0.60, 0.74→0.55), and in one case, decreased transiently, then normalized. The reason for this phenomenon is unclear. Platelet count was within normal levels during the treatment.

Next, we discuss the changes in certain parameters during the course of this study in three patients (III-1, III-2, III-5) treated with 200 ml of SNMC daily.

Minor changes in the mitogenic responsiveness of lymphocytes to PHA, Con A and PWM were detected in one case but not in the others. These changes seemed to be within the normal range (Fig. 1). Serum Na slightly increased immediately after initiation of the treatment and then decreased to normal levels. On the other hand, serum K showed an inverse pattern. These changes were, however, also within the normal range. The elevated GOT and GPT noted in 2 cases increased slightly 2 weeks after initiation of the treatment and then gradually decreased to normal levels. The reason for this transient elevation is unclear (Fig. 2). Serum lipids (TC and TG) and serum proteins (TP and Alb.) were all within the normal range during the period of treatment (Fig. 3). Immunoglobulin (IgG, IgM and IgA) was normal and unchanged in 2 cases (III-1 and III-2). The elevated value noted in the other case (III-5) gradually lowered to near normal levels. Serum γ-globulin in this case was also high initially but
lowered to almost normal levels (2.53 g/100 ml → 1.93 g/100 ml) during the treatment (Fig. 4). The levels of BUN, Cr and UA also tended to decrease during the period of daily treatment. These changes were, however, also within the normal range (Fig. 5).

The following are the results of a case of hemophilia A (AC, 24-year-old male, F VIII: C 1.0%) administered 400 ml SNMC daily, the largest dose of SNMC ever used in Japan.
Fig. 4. Changes in immunoglobulin after intravenous administration of SNMC (200 ml).

Fig. 5. Changes in renal function after intravenous administration of SNMC (200 ml).
Serum electrolytes (Na and K), renal function (BUN, Cr and UA) and liver function (GOT and GPT) were all within normal ranges and remained unchanged during the treatment (Fig. 6). The mitogenic responsiveness of lymphocytes to PHA, Con A and PWM was slightly lowered after 2–3 weeks, but not significantly. Skin tests (MULTITEST CMI®) were positive and remained unchanged throughout the period of this study (Fig. 7). Serum proteins (TP and Alb.), lipids (TC and TG) and immunoglobulin (IgG, IgM and IgA) were within the normal range (Fig. 8). OKT4/OKT8 ratio was elevated from 0.37 (before treatment) to 0.74.
Fig. 8. Changes in serum lipids and immunoglobulin after intravenous administration of SNMC (400 ml).

Fig. 9. Changes in lymphocyte subset after intravenous administration of SNMC (400 ml).
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after 1 week and fell to 0.59 after 2 weeks, then reached a maximum value of 0.80 after 3 weeks. The ratio gradually decreased along with the reduced frequency of SNMC injections. It seems that this phenomenon reflects a fluctuation in the OKT4 lymphocyte count, which increased dramatically from 513/mm³ to 1141/mm³, which is within the normal range. NK cell activity, however, was unchanged and remained low. RBC and platelet counts were within normal levels, however, WBC count increased transiently with treatment of dental caries (Fig. 9).

Another case (III-10) received long term treatment over a 40-week period (Fig. 10). The patient was 29-year-old male with hemophilia A whose factor VIII activity was below 1.0%. He was an AC of HIV. He complained chiefly of slight hemarthrosis of the right elbow joint and occasionally of the ankle joint. The patient, a physically well developed man, had been receiving intravenous injections of factor VIII concentrate 4-6 times per month, which was relatively less frequent. While the deficiency in factor VIII was severe, the clinical effects were mild in this case.

OKT4/OKT8 ratio in this case was 0.22 one year before and 0.38 at the beginning of this study. At first, 40 ml of SNMC was injected daily, then subsequently increased to 100 ml after 8 weeks. OKT4/OKT8 ratio gradually rose from 0.38 to 0.65 by the end of the 12th week. It fell again, however, to 0.4 by the 21st week, hence, the patient was then given 200 ml of SNMC every day in accordance with the protocol of the Japan National Research Committee. OKT4/OKT8 ratio rose to 0.6 but again fell to 0.5 after the 25th week upon administration of SNMC every second day. After that, the ratio again rose to 0.6 by the 27th week with daily administration of SNMC. Finally, it was found that

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Fig. 10. Changes in lymphocyte subset during long term administration of SNMC.
the OKT4/OKT8 ratio could be maintained from 0.55 to 0.6 by daily administra-
tion of 200 ml of SNMC, the level falling when the dose of the agent was decreased. 
Although some slight fluctuations were noted, the number of OKT4 lymphocytes 
was almost always high. OKT4/OKT8 ratio was maintained from 0.5 to 0.65 as 
of the 40th week and no side-effects, such as a decrease serum K, were observed 
throughout the period of this study. Liver dysfunction observed in this case 
dramatically improved not only with respect to GOT, GPT and γ-GTP but also 
TTT and ZTT. The patient is now clinically in good health.

**DISCUSSION**

In our clinical trial, an increase in the number of OKT4 lymphocytes was 
observed in 8 out of 9 AC patients during administration of GL. The OKT4 
lymphocyte count, however, remained virtually unchanged in the HIV-negative 
hemophilia patient group. The increase in OKT4 lymphocytes may be due to an 
anti-HIV effect of GL and the effects of a biological response modifier (BRM) 

GL, at a concentration of 0.5–1.0 mg/ml, completely inhibited HIV replica-
tion in vitro; 50% inhibition was obtained at a concentration of 0.125 mg/ml of 
GL (Ito et al. 1987). If these concentrations could be maintained in vivo, HIV 
infection could well be eradicated. This is the purpose of large dose administra-
tions of SNMC.

The BRM activity of GL was mainly evaluated according to increases in the 
OKT4 lymphocyte count. An important and significant problem, however, is 
whether the increase in OKT4 lymphocytes is derived from normal multipotential 
stem cells in the bone marrow or from infected lymphocytes. If the increase in 
OKT4 lymphocytes were derived from HIV-positive cells, an aggravation of 
clinical symptoms could be expected: AC and ARC would develop into ARC and 
AIDS, respectively. Nevertheless, one patient who underwent long term treat-
ment has showed no such deterioration. This suggests, therefore, that the newly 
produced OKT4 lymphocytes are derived from normal stem cells, though this 
remains unproven. If these levels of OKT4 lymphocytes can be maintained, or if 
re-administration of SNMC can be relied upon to increase the level of OKT4 
lymphocytes with each successive dose, immunological abilities may be well 
preserved and the development in the disease may be resisted. The 400 ml daily 
dose used in this one case, though still insufficient, seems to be a step in the right.

Meanwhile, liver dysfunction, observed in 70–80% of hemophilia patients 
treated with imported (mainly from the USA) Factor VIII and IX concentrates, 
seems to be ameliorated with SNMC. Improvement in liver dysfunction is of 
great importance in the treatment of hemophilia patients since there exists an 
extremely high incidence of hepatitis B and non A, non B hepatitis with the use 
of Factor VIII and IX concentrates (Mori 1987).

In conclusion, GL administration, which has been shown to increase the
number of OKT4 lymphocytes, should be considered a suitable treatment for preventing the development of AC in hemophilia patients into ARC or AIDS.

References