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Immunomodulatory treatment of HBsAg positive chronic hepatitis with specific substance Maruyama (SSM)

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Summary

Twenty-six patients with HBsAg positive chronic hepatitis were treated for over 6 months with specific substance Maruyama (SSM) extracted from the cell wall of human tubercle bacilli.

HBeAg was found to disappear in 6 out of the 14 patients (42.9%) with positive HBsAg 10.0 ± 7.4 months after the treatment began, and HBe seroconversion was found in 4 out of the 6 cases. A decrease of more than 2^2 in the anti-HBc titer (IAHA) was observed in 9 out of 11 patients (82%) positive for HBeAg, and in 2 out of 7 patients (29%) negative for HBeAg. None of the patients became negative for HBsAg, although a fall in titer was found in 3 out of 10 HBeAg positive patients. A transient rise in SGOT and SGPT coincident with disappearance of HBeAg was found 3 or 6 months after the start of treatment. These results suggest that SSM induces an immunomodulatory response and has a beneficial effect on HBsAg positive chronic hepatitis.

Key words : hepatitis B, hepatitis B surface antigens, hepatitis B e antigens, DNA polymerase, immunotherapy

Introduction

In 1978, Brzosko¹⁾ treated children with HBsAg positive chronic active hepatitis by administering Bacillus Calmette Guerin (BCG), a substance extracted from the Bovine tubercle bacillus, and found that HBsAg disappeared in about 30% of the cases. Thereafter, Bassendine²⁾ reported that immunotherapy with BCG was effective in 3 out of 9 patients with total elimination of HBV associated antigens or decreased viral replication accompanied by decreased inflammatory activity.

Specific substance Maruyama (SSM)³⁾ is a substance extracted from human tubercle bacilli (Aoyama B strain). It has been reported that SSM activates cellular immunity^{4,5)} and stimulates interferon production⁶⁾. Based on experimental^{7,8)} and clinical^{9,10)} studies, Maruyama et al. reported beneficial therapeutic responses in malignant tumors, although a

consensus was not yet been reached worldwide.

In 1978, we commenced studies on the effectiveness of treatment with SSM for HBsAg positive chronic liver diseases. Here, we will report on the effects of SSM on both liver biochemistry and HBV replication as evidenced mainly by the HBe antigen-antibody system.

Subjects and Methods

Twenty-six patients with HBsAg positive chronic hepatitis were studied (Table 1). Diagnoses were confirmed histologically for all patients. Fourteen patients (No. 1~14, average age 35.0 ± 10.9 years) were positive for HBeAg, while 12 patients (No. 15~26, average age 37.6 ± 10.5 years) were negative, and five of them were positive for anti-HBe.

HBsAg was estimated by the RPHA method, anti-HBs by the PHA and/or RIA methods, anti-HBe by the IAHA method, and HBeAg and anti-HBe by micro-ouchterlony and/or the RIA method. DNA polymerase (DNAP) was measured by a modification of the method of Kaplan⁽¹⁾.

Treatment with SSM consisted of intramuscular injection of two solutions, A and B (a 1:10 dilution of solution A). Eleven patients, No. 1~3 and 15~22, were injected every other day with A and B solutions of SSM alternately, while the other patients were injected

Table 1 Summary of patients

No.	Name	Age	Sex	Histological diagnosis	HBsAg (RPHA)	anti-HBs (PHA)	HBeAg (MO or RIA)	anti-HBe (MO or RIA)	anti-HBe (IAHA)
1	Y.S.	31	M	CLH	2 ⁹ <	(-)	(+)	(-)	2 ¹² <
2	N.K.	36	M	CPH	2 ⁹ <	(-)	(+)	(-)	2 ¹² <
3	H.M.	43	M	CPH	2 ⁹ <	(-)	(+)	(-)	2 ¹² <
4	K.U.	41	F	CH(2 B)	2 ⁹	(-)	(+)	(-)	2 ¹² <
5	S.H.	54	M	CH(2 A)	2 ¹⁴	(-)	(+)	(-)	2 ²⁰ <
6	T.O.	18	M	CPH	2 ⁹	(-)	(+)	(-)	2 ¹⁷
7	A.N.	43	F	CH(2 B)	2 ¹⁵	(-)	(+)	(-)	2 ¹²
8	Y.K.	39	M	CH(2 B)	2 ⁴	(-)	(+)	(-)	2 ²⁰ <
9	S.Y.	27	F	CH(2 A)	2 ⁷	(-)	(+)	(-)	2 ¹⁹
10	R.M.	51	F	CH(2 B)	2 ⁹ <	(-)	(+)	(-)	2 ¹² <
11	Y.H.	30	M	CPH	2 ¹⁶	(-)	(+)	(-)	2 ¹⁵
12	H.I.	22	M	CH(2 A)	2 ¹¹	(-)	(+)	(-)	2 ¹²
13	K.O.	22	M	CH(2 A)	2 ⁹	(-)	(+)	(-)	2 ¹³
14	Y.T.	33	M	CPH	2 ¹²	(-)	(+)	(-)	2 ²⁰ <
15	H.K.	37	M	CH(2 B)	2 ⁹ <	(-)	(-)	(-)	2 ¹¹
16	T.N.	32	M	CH(2 B)	2 ⁹ <	(-)	(-)	(-)	2 ¹⁰ <
17	Y.F.	44	M	CPH	2 ⁹ <	(-)	(-)	(-)	2 ¹¹ <
18	H.H.	30	M	CH(2 A)	2 ⁹ <	(-)	(-)	(-)	2 ¹¹ <
19	K.Y.	28	F	CH(2 A)	2 ⁹ <	(-)	(-)	(+)	2 ¹¹ <
20	H.K.	50	F	CPH	2 ⁷	(-)	(-)	(+)	2 ⁷
21	T.T.	46	M	CPH	2 ⁵	(-)	(-)	(+)	2 ¹² <
22	R.I.	47	M	CH(2 A)	2 ⁹	(-)	(-)	(+)	2 ⁷
23	E.N.	55	M	CH(2 B)	2 ⁵	(-)	(-)	(+)	2 ⁷
24	Y.K.	32	M	CPH	2 ⁴	(-)	(-)	(-)	2 ⁷
25	A.Y.	28	M	CH(2 B)	2 ⁷	(-)	(-)	(-)	2 ¹³
26	E.H.	22	M	CH(2 B)	2 ¹⁰	(-)	(-)	(-)	2 ²⁰ <

CLH : chronic lobular hepatitis, CPH : chronic persistent hepatitis, CH (2 A) : chronic aggressive hepatitis (moderate), CH (2 B) : chronic aggressive hepatitis (severe)

with solution A twice a week.

The subjects were treated with SSM for at least 6 consecutive months, the mean period of treatment being 13.6 ± 6.8 months in the HBeAg positive group with actual periods of treatment ranging from 6 to 28 months, and 19.8 ± 10.5 months in the HBeAg negative group with actual periods of treatment ranging from 6 to 45 months. However, in two HBeAg positive cases (No. 12 and 13) who showed marked elevation of transaminase after 3 and 4 months of treatment respectively, injections were suspended for two or two-and-a-half months until they return to almost normal or pretreatment levels.

In four HBeAg negative cases (No. 16, 18, 19 and 20), SGOT and SGPT fell and injections were suspended when they reached normal levels. Observations of HBV seromarkers and biochemical observations were, however, subsequently made. Including these cases, the mean period of observation was 16.3 ± 8.9 months. Serum markers of HBV and transaminases were monitored at intervals of about 4 weeks throughout the period of the trial.

Results

1. Changes in HBV seromarkers

As shown in **Fig. 1**, HBeAg disappeared in 6 out of 14 HBeAg positive patients (42.9%), treated for 10.0 ± 7.4 months. Three of these 6 patients had chronic persistent hepatitis, one had chronic aggressive hepatitis, moderate, and two chronic aggressive hepatitis, severe. The disappearance of HBeAg was not associated with the histological diagnosis before treatment. Of the 6 patients, 4 produced persistent anti-HBe (seroconversion). The other 2 patients developed anti-HBe, but it soon disappeared.

One of the 7 patients in the HBeAg negative group who were negative for anti-HBe before treatment became positive for the antibody after treatment.

Fig. 1 Results of serial testing for HBeAg and anti-HBe in 14 patients with chronic type B hepatitis

No patients became negative for HBsAg in either the HBeAg positive or negative groups. In 3 out of the 10 HBeAg positive patients for whom comparisons of the HBsAg titers before and after treatment had been made, a fall of more than 2^2 in the titer was found using the RPHA method. In the remaining cases in the HBeAg positive group and in all seventeen cases in the HBeAg negative group, the titer of HBsAg remained almost unchanged.

Of the 21 patients who had been negative for anti-HBs before treatment, 4 became positive for anti-HBs, 2 each from the HBeAg positive and negative groups.

As for changes of more than 2^2 in the anti-HBc titers (IAHA) before and after treatment, a decrease was observed in 9 out of the 11 patients in the HBeAg positive group (82%) and in 2 out of the 7 patients in the HBeAg negative group (29%).

Changes in DNAP in time course was measured in eight patients positive for HBeAg. The mean level of DNAP (cpm/ml) was 615 ± 439 ($n=8$) before treatment, 678 ± 560 ($n=7$) at 2 months, 841 ± 637 ($n=4$) at 4 months, 844 ± 876 ($n=7$) at 6 months, 2027 ± 3059 ($n=4$) at 9 months, and 245 ± 51 ($n=4$) at 12 months after the beginning of treatment. In one patient, DNAP activity was 768 before treatment, 1358 at 4 months, 20400 at 5 months, 1190 at 6 months, 177 at 9 months, and 180 at 12 months, showing a distinct decrease coincident with a transient rise of transaminases and the disappearance of HBeAg (Fig. 2).

2. Changes in serum transaminase levels

The changes in the SGOT levels of the 14 patients in the HBeAg positive group over the observation period are shown in Fig. 3. A transient rise in SGOT was seen 3 months after the beginning of treatment in two patients and 6 months after the beginning of treatment in

Fig. 2 Clinical course of case 8

Fig. 3 Changes of serum GOT in 14 patients with positive HBeAg

Fig. 4 Changes of serum GPT in 14 patients with positive HBeAg

6 patients. Calculations for the HBeAg positive group as a whole revealed that the SGOT level at 9 months was significantly lower than that measured before treatment, and 3 and 6 months after treatment.

SGPT levels exhibited changes similar to SGOT and a transient rise was observed in 3 and 7 patients 3 and 6 months after treatment respectively (Fig. 4). In the HBeAg negative group, however, no such rise in either SGOT or SGPT was observed except in one case (Fig. 5, 6).

3. Histological changes of the liver (Table 2)

The hepatic tissue was obtained in five patients by performing needle liver biopsy twice. The second biopsy was performed 5.2 ± 0.8

Fig. 5 Changes of serum GOT in 12 patients with negative HBeAg

Fig. 6 Changes of serum GPT in 12 patients with negative HBeAg

Table 2 Changes of histological diagnosis during SSM treatment

No.	Name	1st biopsy (at entry)	Interval (Month)	2nd biopsy	HBeAg
1	Y.S.	CLH	5 M	CPH	+
10	R.M.	CH(2 B)	6 M	CH(2 B)	+
13	K.O.	CH(2 B)	5 M	CH(2 A)	+
16	T.N.	CH(2 B)	4 M	CH(2 A)	—
26	E.H.	CH(2 B)	6 M	CH(2 B)	—

CLH : chronic lobular hepatitis

months after the beginning of treatment. Of the five patients, three were positive and two negative for HBeAg. In the former three patients, the histology of liver specimens was improved in two (one of whom revealed a seroconversion of HBeAg) and unchanged in one. In the latter two patients, it was improved in one and unchanged in the other.

4. Side effects

No remarkable side effects caused by the treatment were observed in any of the patients.

Discussion

In the past, one therapy used with cases of chronic hepatitis due to HBV infection has attempted to modulate the immune response to the virus on the basis of the hypothesis that the pathological features result from the host immune response, though these attempts have been for the most part unsuccessful¹²⁾. Among these attempts, treatment with BCG^{1,2)} was reported to have therapeutic possibilities in patients with HBsAg positive chronic active hepatitis, though follow-up tests have yet to be performed.

In 1978 we had already started treatment with SSM in patients with HBsAg positive chronic hepatitis in an attempt to modulate immune responses. The above-mentioned reports on BCG^{1,2)} spurred us on to further studies of additional cases of patients with HBeAg positive chronic liver diseases. Such patients made good subjects because there is a close association between HBe antigenemia, the presence of Dane particles and DNA-polymerase, and a high level of contagiousness, and because the disappearance of HBeAg and its seroconversion into anti-HBe can be used as an index of successful treatment.

In the present study, 6 out of 14 HBeAg positive patients (42.9%) lost HBeAg during an average period of treatment of 10.0 ± 7.4 months. Four of the patients (28.6%) produced anti-HBe which persisted (so-called seroconversion). In addition, 1 out of the 7 patients negative for anti-HBe in the HBeAg negative group became positive for anti-HBe after treatment. These results suggest that the SSM induced an immunomodulatory effect and a reduction in viral replication in HBsAg positive chronic hepatitis.

Spontaneous seroconversion of HBeAg, especially in patients with chronic liver disease, occasionally occurs in the natural course of the disease. Spontaneous annual seroconversion rates from HBeAg to anti-HBe can be calculated from a few published studies and vary from less than 5% in untreated British patients¹³⁾ to 17% in untreated Taiwanese pati-

ents¹⁵⁾. In a Japanese report, the annual rate of patients becoming negative for HBeAg was calculated to be 9.6%¹⁵⁾. Compared with data for the natural course of the disease, the seroconversion rate with SSM is superior and the median time between the start of therapy and the disappearance of HBeAg (10.0 months) is shorter, suggesting that the seroconversion or disappearance of HBeAg in the present study was related to treatment with SSM.

The rate of HBeAg seroconversion after treatment was reported to be 27.3% with prednisolone and/or azathiopurine¹⁶⁾, 75% with levamisole¹⁷⁾, 18.2% with Ara-A¹⁸⁾, 33.3% with transfer factor¹⁹⁾, 4.5% with interferon²⁰⁾ and 22.2% with OK-432²¹⁾. The rate with SSM (28.6%) is comparable to these results. In addition to HBeAg seroconversion, the titer of anti-HBc tended to decrease after the treatment with SSM, suggesting that SSM also has further beneficial effects with regard to HBV replication, though these were not always pronounced and further studies with more cases are requested.

The disappearance of HBsAg was not seen in any of the patients treated with SSM. On the other hand, anti-HBs, the neutralized antibody for HBV infection, developed in four (15.4%) out of the 26 patients in the present study. It is difficult to conclude that the appearance of anti-HBs resulted from the effects of SSM, since the anti-HBs level before treatment in some of the patients had been measured according to the PHA method and not by the RIA method. Accordingly, the possibility remains that anti-HBs would have been positive even before the treatment in these patients if it had been measured by RIA.

A reduction in DNAP is considered a favorable effect in the treatment for HBV infection¹²⁾, since it indicates a decrease in viral replication. In the present study DNAP tended to decrease after at least twelve months of treatment. However, a transient rise before this reduction was also observed, as shown in the typical case outlined in **Fig. 2**. On these changes in DNAP, no clear explanation is yet possible. As far as immunomodulating therapy is concerned, no data is as yet available on the effects on DNAP.

A transient rise in SGOT and SGPT coincident with the disappearance of HBeAg was observed 3 or 6 months after treatment with SSM in 8 patients out of the HBeAg positive group. It was followed by a significant fall from pretreatment levels after 9 months. Similar results have been reported elsewhere^{16, 22)}, where the termination of therapy with prednisolone was followed by a transient rise in serum aspartate transaminase, coincident with disappearance of HBeAg and seroconversion. Furthermore, some reports^{14, 22)} have disclosed that a transient rise in transaminases is followed by HBeAg seroconversion in the natural course of the disease. These results allow us to consider the possibility that a transient rise in transaminases may be a condition for the seroconversion of HBeAg. These events may represent development of a state of enhanced immunity to the virus.

SSM is almost free from any side effects. This is the main advantage of SSM compared to other treatments, including corticosteroid and antiviral drugs.

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