J. Trad. Med. 24, 59－66, 2007

Medical and Pharmaceutical Society for WAKAN-YAKU

Effect of a traditional Chinese medicine, maobushisaishinto, on the antibody titer after influenza vaccination: A randomized, placebo-controlled, double-blind trial

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Background: It was shown that a traditional Chinese medicine, maobushisaishinto (MBST), had adjuvant effects on influenza vaccination in an animal experiment and an open trial in elderly subjects.

Purpose: To examine the adjuvant effects of MBST in a closer clinical experiment.

Methods: Forty-seven healthy subjects between 20 and 71 y of age were randomly assigned to two groups (control and MBST groups) in a double-blind manner. The subjects in the MBST group (n=23) took 1.68g spray-dried powder of MBST/day for two weeks; the rest (control subjects, n=24) took the same amount of indistinguishable placebo. Then subjects were vaccinated against influenza viruses (A/H1N1, A/H3N2 and B). Serum hemagglutination inhibition titers were measured at weeks 0, 1, 2, 4, and 12.

Results: The titers against the three viruses were increased continuously for the first two weeks and leveled off. However, MBST was not superior to placebo in any titers.

Conclusion: We could not find any adjuvant effects of MBST in this experimental condition.

Key words adjuvant, maobushisaishinto, influenza vaccination, randomized clinical trial.

Introduction

As of this writing, avian influenza A (H5N1) virus is not highly contagious among humans yet. However, it is postulated that it will transform and become highly contagious in the near future, and that a disastrous pandemic will follow. In order to prevent such a pandemic, the development of safe and effective vaccines is likely to be the single most important public health tool[1] in view of the reported resistance of influenza A (H5N1) virus to antiviral agents.[2] The effects of a vaccine against influenza A (H5N1) virus were recently reported.[3] However, its immunity was poor to moderate at best,[1] considering that the vaccine required two doses of 90μg per person.[3] Under such circumstances adjuvant studies for influenza vaccination are very urgent. We have recently tested whether hochuekkito has any adjuvant effects in the case of influenza vaccination. However, hochuekkito did not show any beneficial effects for antibody titers in serum.[4]

Maobushisaishinto (MBST), a traditional Chinese medicine, has been used to treat weakness or common cold, especially for the elderly.[5,6] Takagi *et al.*[7] reported that IgG antibody formation, which deteriorates with age, against two kinds of thymus dependent antigen, dinitrophenylated keyholelimpet hemocyanin and bacterial α-amylase, was enhanced in mice with MBST. They also found that the number of IgM antibody-producing cells in the thymus in mice was enhanced when MBST was administered for two weeks prior to antigen (dinitrophenylated dextran) challenge. The ratios of enhancement appeared to be higher in mouse groups of 24, 52 and 78 weeks of age than in a group of 10 weeks of age, although the statistical analysis was not performed.[8] MBST enhanced antibody production against influenza virus B strain after vaccination with influenza hemagglutinin (HA) vaccine in one-year old mice.[9] Oral administration of MBST increased the survival rates and mean survival period of mice after infection with influenza virus.[10] Iwasaki *et al.*[11] conducted a randomized control study of MBST in eighteen elderly subjects with influenza vaccine, and claimed that MBST had adjuvant effects 4 weeks after vaccination. In the present study, we examined the effect of MBST in a closer clinical test; namely a placebo-controlled, double-blind test using 47 healthy volunteers as subjects and measuring titers at several time points.

Subjects

One hundred and six healthy volunteers were recruited from local companies in Toyama Prefecture for the present study. They had not been taking any herbal medicine, hormone therapy or non-steroidal anti-inflammatory drugs during the previous 3 months. Volunteers who had a history of allergy to eggs or of adverse effects after vaccination were not eligible. Fifty-seven subjects were not able to enter the study because their antibody titers against A/H1N1,

A/H3N2 or B were over the clinically effective antibody titers (128 times,[12]). The remaining 49 subjects, whose titers were all 80 times or below, were randomly allocated either to the MBST group (n=24) or to the control group (n=25) in a double-blind manner. One MBST subject and another in the control group were diagnosed with influenza during the study period, so their data were excluded, and the remaining 23 and 24 subjects in the MBST and placebo groups, respectively, were analyzed (Table 1). Of the 47 subjects analyzed, two MBST and another two control subjects were diagnosed with influenza during the last influenza season. Three and four subjects in the MBST and placebo groups, respectively, were vaccinated last year (Table 1).

Methods

Subjects in the MBST group took 6 MBST capsules per day from day -14 to -1, and those in the control group took indistinguishable placebo capsules. On day 0 (week 0), unconsumed test capsules were returned for calculation of compliance, and all subjects were vaccinated during the last 10 days in November before an influenza epidemic might begin. Blood samples were taken at weeks 0, 1, 2, 4, and 12. Serum samples were kept frozen at -80℃ until analysis. Antibody titers to hemagglutinin were measured by a standard microtiter hemagglutination inhibition method with chicken erythrocytes (Toyo-Bio Inc., Tokyo).[13] The strains used as antigens were A/New Caledonia/20/99 (H1N1), A/New York/55/2004 (H3N2) and B/Shanghai/361/2002, which were the same strains as the vaccines used in the present study. At week 0, study subjects were asked if they had experienced any adverse effects with test capsules and asked to guess which test capsules they had been taking. Subjects were asked to maintain their body weights and physical activity levels and to consume their habitual diets during the whole study period. The present study was approved by the ethics committee of Toyama Medical and Pharmaceutical University (the name used before joining University of Toyama), and written informed consent was obtained from each participant.

The vaccine of a lot number (Lot 304-A in 2005) used in the present study contained 30μg of each HA antigen in 1ml: A/New Caledonia/20/99 (H1N1), A/New York/55/2004 (H3N2), B/Shanghai/361/2002 (Denka Seiken Co., Ltd., Niigata). A subcutaneous injection of 0.5ml of the vaccine was given once in the afternoon (between 12:00 and 13:00). MBST and placebo capsules were kindly donated by Kotaro Pharmaceutical Co., Ltd. (Osaka). The active ingredient (1.68g) of MBST capsules was manufactured as spray-dried powder of hot water extracts from Ephedra Herb 4.0g, Asiasarum Root 3.0g and powder of Aconite 1.0g. A fingerprint of MBST (Lot number: Z0516B, the same lot used in the present study) is shown in Fig. 1. Placebo consisted mainly of cane sugar and no herbal medicine. Both groups of subjects took 2 capsules of test capsules three times daily (before breakfast, lunch and supper) for 14 days.

Table 1 Characteristics of subjects

	Placebo	Active	p
Number	24	23	
Male	7	8	0.69
Average years of age	36.4±11.0	37.9±13.1	0.75
Number of younger subjects (<40 years old)	15	14	0.91
Number of smokers	7	15	0.013
Number of subjects diagnosed with influenza last year	2	2	0.97
Number of subjects vaccinated last year	4	3	0.73

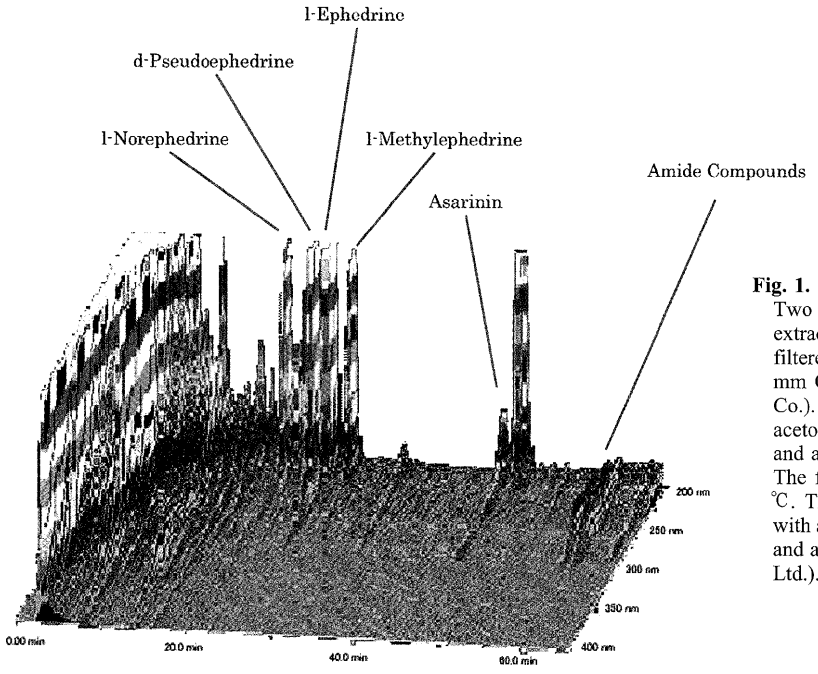

Fig. 1. Fingerprint of MBST by HPLC
Two g of MBST (Lot # Z0516B, used in the present study) was extracted with 50mL of 50% methanol under ultrasonication. A filtered MBST sample (20μl) was applied to a 5μm 4.6×250 mm ODS column (Chemcosorb 300-5C18, Chemco Scientific Co.). The elution sequence was: 20mM lauryl sodium sulfate / acetonitrile / phosphoric acid (1625:875:1) for the first 40min and a linear gradient to acetonitrile / water (11:9) over 50min. The flow rate was 1.0mL/min, and the column was set at 30 ℃. The UV data ranging from 200 to 400nm were collected with a photodiode-array (PDA) detector (L-7450, Hitachi, Ltd.), and analyzed with a system analysis software (D-7000 Hitachi, Ltd.).

Data were analyzed on a per protocol base, and are expressed as means±S.D. Negative titer values (<10) were arbitrarily regarded as 5. Titers were transformed to a logarithmic scale, and geometric means were used for further calculations. The results were analyzed by conventional statistical methods (repeated measure ANOVA for geometric mean titers [GMT]). Chi-squared test was used for the comparison of subjects' guess about the randomization status. A P value <0.05 was considered to indicate statistical significance. StatView software (SAS Institute, Cary, North Carolina) was used for statistical analysis.

Results

No subjects complained of any serious adverse effects of test capsules throughout the whole study period, and all 47 subjects completed the study. Table 1 shows the characteristics of subjects. There were no significant differences between groups except that the number of smokers in the MBST was significantly larger than in the control group. The subjects in the placebo group guessed that their capsules were MBST (n=3), unclear (n=21) and placebo (n=1); those in the MBST group guessed that their capsules were MBST (n=6) unclear (n=16) and placebo (n=2), not significant by chi-squared test between groups (p=0.18). Compliance with the capsule administration schedule was more than 90% in both groups.

Figs. 3-5 show changes after vaccination in serum anti-A/H1N1, anti-A/H3N2 and anti-B virus HA antibody titers, respectively. There were no significant differences between groups except that anti-A/H3N2 virus HA antibody titers in the control group were significantly higher than in the MBST group at week 4 (Fig. 4). Antibody titers were also compared within the older subjects (≥40 years old) alone or the younger alone (<40 years old), and within the smokers alone or the non-smokers alone. Those subgroup comparisons did not show any significant differences between the MBST and control groups (Figs. 3-5). Comparisons

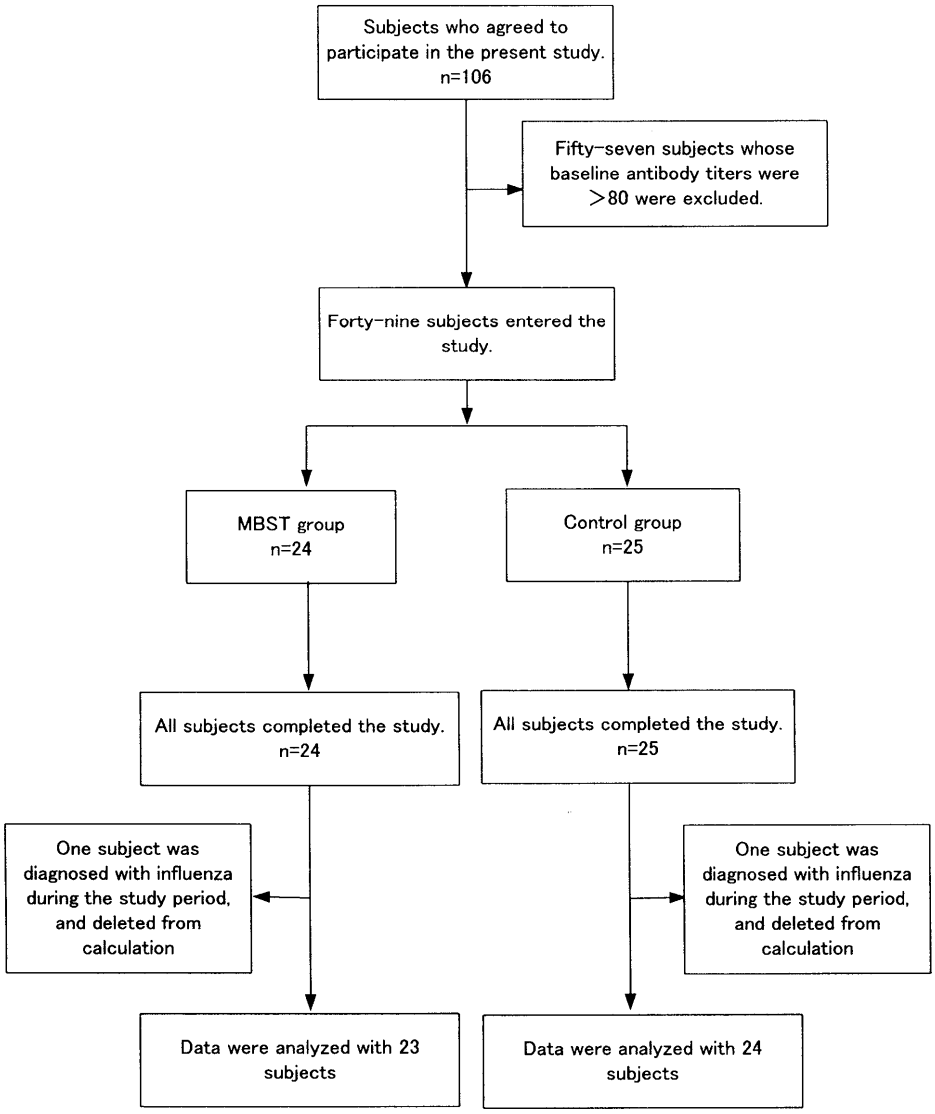

Fig. 2. Flow chart of subject recruitment and trial profile

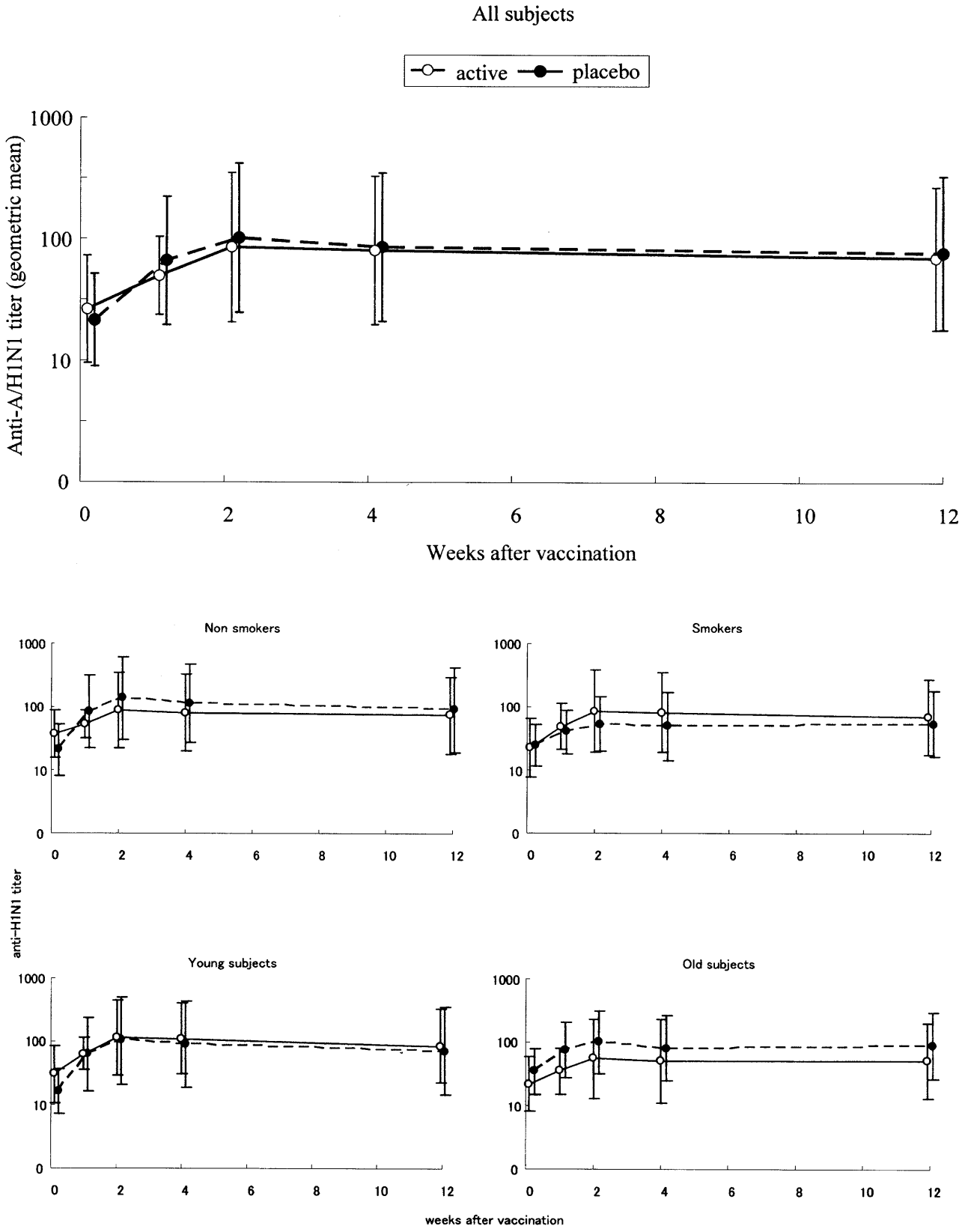

Fig. 3. Changes in serum anti-influenza virus A/H1N1 HA antibody titers after vaccination
Each value represents the mean ± S.D.

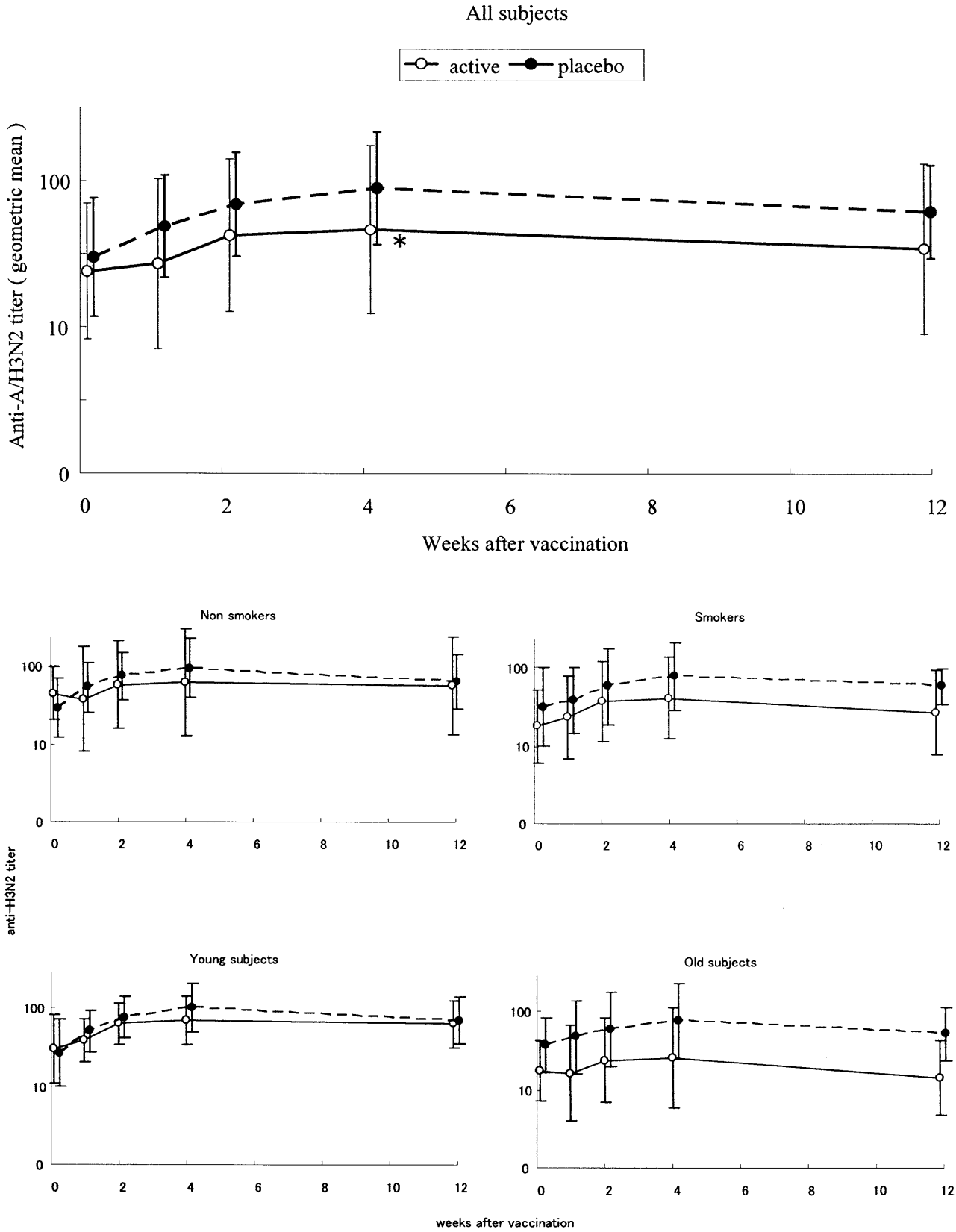

Fig. 4. Changes in serum anti-influenza virus A/H3N2 HA antibody titers after vaccination
Each value represents the mean ± S.D. *$p<0.05$.

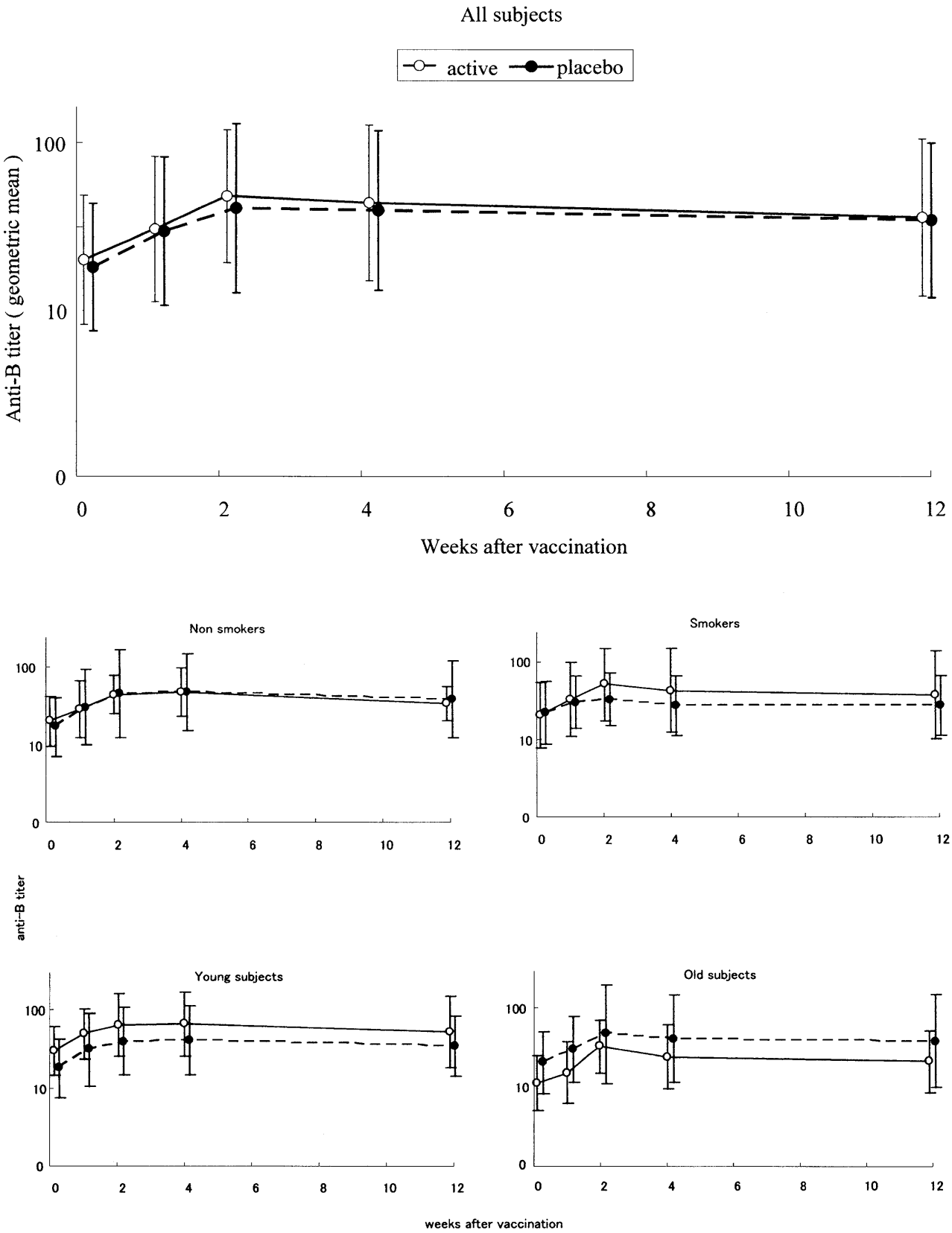

Fig. 5. Changes in serum anti-influenza B virus HA antibody titers after vaccination
Each value represents the mean ± S.D.

between smokers and nonsmokers or between older and younger subjects did not show any significant differences either within the MBST group alone or within the control group alone (data not shown). Subanalysis with those subjects whose titers were less than 10 (n=14 for A/H1N1, n=15 for A/H3N2 and n=18 for B) did not show any significant or substantial differences between groups either (data not shown).

Discussion

It seems that an avian H5N1 influenza virus was accumulating mutations during human-to-human transmission in Indonesia.[14] It is only a matter of time that one of the avian H5N1 influenza viruses currently found in many areas in the world will actually cause the next influenza pandemic. Vaccination is the main preventive measure for avian influenza viruses, and there will be a shortage of vaccine. It is, therefore, very important to find an appropriate medicine that has adjuvant effects on vaccination.

In the present study, the increase in titers after vaccination in the MBST group was not superior to the control group, although it was shown that MBST had adjuvant effects on influenza vaccination in animal experiments and also in a randomized controlled study in elderly persons. As written in Introduction, Iwasaki *et al.*[11] conducted a controlled trial of MBST in only eighteen elderly subjects. Unfortunately their control group did not have any placebo. They compared the ratios of the attained titer values at week 4 divided by baseline, and found that the average ratio of anti-A/H3N2 virus HA antibody titers was significantly higher in the MBST group than in the control group. However, they did not show the actual titers at week 4, but only the ratios. Besides, the baseline titers were described only as <10 in both groups. No titer data were available before or after week 4.[11] Consequently, it is impossible to understand the real difference between groups. Clinical studies related with immunological competence should not be performed in open trials because placebo effects may be formidable.[15]

We administered capsules to study subjects for the two weeks prior to vaccination. This method was adopted according to the animal experiments of Takagi *et al.*[8] We did not use the method of Iwasaki *et al.* (MBST administration for the week prior to vaccination and for another week afterward),[11] because their results were rather weak as described above.

It is not clear why the titer of anti-A/H3N2 virus HA antibody in the MBST group was lower than in the control group at week 4. It is possible that the difference might be found by chance, taking into consideration the number of statistical calculations.

Unfortunately there was a significant difference in the ratio of smoking status between groups. However, as described in Results, there were no significant differences in any titers between smoking and non-smoking subgroups, or between the MBST and control groups within the smokers or nonsmokers. It seems, therefore, unlikely that smoking had any effects on our results. It is also the case for the older and younger subgroups.

In conclusion, we could not find any adjuvant effects of MBST. Further research to find materials with adjuvant effects is urgently needed.

Acknowledgement

This study was supported in part by Polyene Project Corporation and Mr. Kanji Nakajima. We are grateful to Ms. Hiroko Hamatani and Ms. Shizuko Takebe for their technical assistant. We are also grateful to Mr. Kouichi Watanabe for his technical support and valuable information. One of the present authors (MH) was a post-doctoral fellow of the Center of Excellence program (No J07).

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Japanese abstract

これまでに動物実験や高齢者に対する非盲検試験で，麻黄附子細辛湯のインフルエンザワクチンに対するアジュバント効果が報告されているが，より精度の高い臨床試験で検討されたことはない。そこで今回，20〜71才の健常者47人を対象として，麻黄附子細辛湯の血清中抗インフルエンザウイルス抗体価に対する影響について検討した。被験者を無作為に実薬群（n=23）とプラセボ群（n=24）に振り分け，二重盲検法にて実薬またはプラセボを2週間投与したところで，ワクチンを接種した。その後採血を0,1,2,4,12週目に行い，上記抗体価を測定した。ワクチン接種後のインフルエンザ抗体価はA型（H3N2, H1N1），B型ともに実薬群で有意に優れているとは言えなかった。今後世界中で鳥インフルエンザのヒトへの感染とその流行が予想され，アジュバント効果がある薬剤の重要性が極めて大きくなる。他のアジュバント効果を持つものの検討が必要である。

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