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TWO DIFFERENT HISTAMINE-SENSITIZING ACTIVITIES OF PERTUSSIS VACCINE OBSERVED IN MICE ON THE 4TH AND 12TH DAYS OF SENSITIZATION

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SUMMARY: The histamine-sensitizing (HS) activities of commercial precipitated diphtheria-tetanus-purified pertussis combined vaccines (DTP) submitted to the national quality control tests during the period from 1980 to 1991 were measured in mice on days 4 (EHS) and 12 (LHS) of sensitization. Annual averages of the EHS activity of the vaccines showed continuous decline during the period, while the LHS activity stayed unchanged except for 1990 and 1991. Correlation analysis between these two HS activities revealed that the vaccines could be differentiated into two groups, those showing a significant correlation and those lacking it, depending on their source manufacturers. When the vaccine was incubated at 37 C for four weeks, both the HS activities increased for the first couple of weeks at different rates, reaching their peaks after different periods; three weeks for the EHS activity and two weeks for the LHS activity. Treating the reference pertussis vaccine (for toxicity tests) with anti-pertussis toxin horse serum neutralized completely the EHS activity but the LHS activity resisted the serum. These findings suggest a possible difference between the two HS activities in their mode of action, therefore, in their roles in possible reactogenicity of the vaccine, and a necessity for separate controls of the two activities.

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INTRODUCTION

Perfentijev and Goodline (1) described enormously increased susceptibility to histamine of the mice having received pertussis vaccine. The activity attracted many workers' attention (2-4) because of its possible relatedness to the post vaccination shock.

The activity has been assayed in mice and expressed as the dose of vaccine sensitizing 50% of animals to death from histamine challenge (HSD₅₀) (5). The assay method, however, was not sensitive or accurate enough to measure quantitatively the activity of an acellular vaccine (6). Ishida et al. (7) developed a new sensitive method based on the change in rectal temperature of the mice injected with the vaccine followed by histamine challenge four days later. The Ishida's method for assaying the HS activity (8) has been promulgated in the Minimum Requirements of Biological Products of Japan since 1981 (9).

In the course of the national quality control of the acellular pertussis vaccines, Iwasa et al. (10) found a curious expression of the histamine-sensitizing (HS) activity, which had been regarded to reach its maximal expression on the fourth day of injecting mice with a pertussis vaccine, reached again its another peak expression on the 12th day.

The present studies were performed to characterize the LHS activity of current commercial DTP vaccines in the context of the vaccine quality control.

MATERIALS AND METHODS

Vaccines: Reference pertussis vaccine for toxicity tests. The reference pertussis vaccine (for toxicity tests) L-1 (reference vaccine) which has been used for the national quality control tests of pertussis vaccines in Japan since 1981 was used throughout the present study as a reference preparation with a known histamine-sensitizing activity (7,8). It was reconstituted in 12 ml of physiological saline and was serially diluted at four-fold intervals from 1 in 1 to 1 in 64 unless otherwise stated.

Commercial vaccines: DTP vaccines, officially referred to as precipitated-purified pertussis-diphtheria-tetanus combined vaccine, subjected to the national quality control tests during the period from 1980 to 1991, were used in the present study.

Anti-pertussis toxin serum: This was a horse serum raised against purified pertussis toxin (PT) and kindly presented by Dr. Wang Cheng-Huai of Lanzhou Institute of Biological Products in China.

Mice: Female mice of four weeks old (Std-ddY, Shizuoka Laboratory Animal Center, Hamamatsu, Shizuoka, Japan) were used. Randomly grouped five mice were housed in each cage containing wood shavings in an animal room at a temperature of 22 to 24 C. They were fed on pellets and provided with water *ad libitum*.

Determination of histamine-sensitizing activities: The HS activity reaching its maximal expression on the fourth day of injection (EHS activity) was measured by the Ishida's method (7,8). In brief, groups of 10 mice each were intraperitoneally injected with a commercial DTP vaccine or a dilution of the reference vaccine in 0.5-ml doses. The mice were each intraperitoneally inoculated with 4 mg of histamine dihydrochloride (Sigma Chemical Co., Ltd., St. Louis, MO) in 0.5 ml of saline four days later. Their rectal temperatures were measured 30 min after the histamine challenge (8).

For measuring the HS activity, showing the peak expression on the 12th day of injection (LHS activity), these mice injected with DTP or the reference pertussis vaccine received the histamine challenge and the rectal temperature measurement 12 days after the vaccination.

Neutralization of the HS activities of the reference pertussis vaccine with anti-pertussis toxin horse serum: The reference vaccine and the anti-PT horse serum were mixed to make their concentrations five histamine-sensitizing units (HSU) per milliliter and 1 in 10 dilution of the serum, respectively. The reference vaccine mixed with physiological saline served as the untreated control. After incubation at 37 C for 2 hr in a water bath, each mixture was intraperitoneally inoculated at 0.5-ml doses into 10 mice of each of 10 groups. One group of mice having received the control, another group having received the serum-treated vaccine, and another group of eight normal mice were challenged with histamine on days two, four, six, eight, 10, 11, 12, 14, 18 and 24 of the sensitization followed by the rectal temperature measurement and mortality recording 30 min later.

Statistic analysis: Analysis of the parallel line assays were carried out by Finney's method. Significance and validity tests were made at a level of $P=0.05$, and confidence intervals were expressed at the 95 % probability level unless otherwise stated.

RESULTS

Changes in Annual Averages of EHS and LHS activities of Commercial DTP Vaccines

The EHS and LHS activities of DTP vaccines submitted to the national quality control tests during the period from 1980 to 1991 were measured and the changes in their annual averages were analyzed. As shown in Fig. 1, the averages of the EHS activities were within the limit of acceptance throughout the period, declining gradually from approximately 0.4 to 0.1 HSU/ml. The averages of the LHS activities were, on the other hand, found to be much higher, being around 3 HSU/ml at the start and stayed unchanged for the first four years. Although the LHS activity showed some drop to the level of approximately 1.5

Fig. 1. Annual averages of EHS and LHS activities of commercial DTP vaccines. EHS (●) and LHS (○) activities of DTP vaccines submitted to the national quality control tests during the period from 1980 to 1991 were measured and their annual averages are presented. Vertical bars represent 95% confidential intervals of the averages.

HSU/ml in 1984 and again to about 0.7 HSU/ml in 1990, it was over the acceptance limit for the EHS activity throughout the period.

Correlation between EHS and LHS Activities of Commercial DTP Vaccines

When the correlation between EHS and LHS activities was analyzed the commercial DTP showed different correlation patterns depending on the source manufacturers. The vaccines from manufacturers A, B and C showed a significant positive correlation ($P=0.01$) between the EHS and LHS activities with a correlation coefficient of 0.534 (Fig. 2-a), while those from manufacturers D, E and F showed no significant correlation ($r=0.229$) between them (Fig. 2-b).

Fig. 2. Correlation between EHS and LHS activities of commercial DTP vaccines. According to correlation analysis between EHS and LHS activities of commercial DTP vaccines, the vaccines could be differentiated depending on their source manufacturers. The vaccines from manufacturers A (○), B (●) and C (△) showed a positive correlation ($p=0.01$) (a), while those from manufacturers D (▲), E (□) and F (■) showed no significant correlation ($p=0.05$) (b) between their EHS and LHS activities.

Different Patterns in Reversion of the Two HS Activities

A vial of DTP vaccine was incubated at 37 C and changes in its EHS and LHS activities were monitored weekly for four successive weeks. The EHS activity which was 0.6 HSU/ml at the start of incubation showed an increase from the second week reaching its peak of about 3 HSU/ml in the third week followed by a rather quick decrease to about 0.6 HSU/ml by the end of incubation.

The LHS activity, on the other hand, was originally as high as approximately 3 HSU/ml, increased to over 10 HSU/ml during the first two weeks, and decreased thereafter to approximately 4.5 HSU/ml by the fourth week, showing a significantly different behavior from that of the EHS activity (Fig. 3).

Fig. 3. Time course of reversion of EHS and LHS activities of a DTP vaccine at 37 C. A vial of DTP vaccine was incubated at 37 C for four weeks and its EHS (●) and LHS (○) activities were monitored. The LHS activity was always higher than the EHS activity, showing a different pattern in the reversion from that of the EHS activity.

*Neutralization of HS Activities of the Reference
Pertussis Vaccine with Anti-PT Serum*

The neutralizing action of the anti-PT horse serum against HS activity of the reference vaccine was examined as described in Materials and Methods. Figure 4 shows the resulting mortalities in each group of the mice on different days of the sensitization shown in the figure.

As seen in Fig. 4, no death was recorded during the first six days in the group injected with the serum-treated vaccine, while mortality over 60% was

Fig. 4. Behavior of HS activity to neutralizing action of anti-PT horse serum. Mortality after histamine challenge of the mice injected with the reference vaccine treated with anti-PT horse serum (○) was compared with that of the mice having received untreated vaccine (●) from the second to the 24th day of injection. Mortality of mice having received only histamine challenge was also shown as a control (□). Each point shows mortality of more than eight mice. Although the serum completely neutralized the HS activity seen in the early stage of sensitization, on around the 10th day it resisted the serum treatment.

noted in the control group during the early stage of the sensitization showing complete neutralization of the EHS activity with the anti-PT serum. Mortalities of both the groups appeared to reach their peaks of 70% on the 10th day of sensitization, suggesting possible resistance of the LHS activity to the serum. They dropped thereafter to 40% in the serum-treated and to around 10% in the control group and increased again gradually to 80% and 60%, respectively, by the end of observation, probably due to some residual influence of the serum treatment.

The rectal temperature measurement after histamine challenge resulted similarly as in the case of mortality. Although the mice having received untreated control vaccine showed significantly lowered rectal temperatures, those having received the serum-treated vaccine showed no drop in their rectal temperatures in the early stage of sensitization. Equally lowered rectal temperatures were noted, however, in both the groups of mice on the 10th day of sensitization in accordance with the drop to the level in the mice having received the serum-treated vaccine.

DISCUSSION

The HS activity of pertussis vaccine had been believed to reach its only peak expression in mice on around the fourth day of sensitization (11) until Iwasa et al. (10) reported the LHS activity, whose maximal expression in mice was on around the 12th day of the sensitization. Although the EHS activity has been a subject of the national quality control of pertussis vaccine since 1981 (9), no quality control measure has ever been applied for the LHS activity.

When annual means of the LHS activity of commercial DTP vaccines were compared with those of the EHS activity, as seen in Fig. 1, the LHS activity of the vaccines was much higher than the EHS activity and these two activities appeared quite different each other in the pattern of change in the annual means. Especially, introduction of the regulation on reversion of the HS activity (EHS activity) of the vaccine in the Minimum Requirements in 1991 resulted in reduction of the LHS activity much more than the EHS activity. This finding showed that the DTP vaccines practically used in Japan might have LHS activity exceeding the allowance level for the HS activity (9) even though the vaccine's EHS activity has been well controlled according to the Minimum Requirements.

To explore the relationship between these two HS activities, correlation analysis was made on the activities of Japanese commercial DTP vaccines. As a result, their correlation patterns were revealed to differ depending on the source manufacturers (Fig. 2), therefore, possibly on the manufacturing procedure of the vaccine. Factors affecting the correlation pattern between the two HS activities has not been identified at present.

A difference was also seen between these two HS activities when a DTP vaccine was incubated at 37 C. Significant reversion of both EHS and LHS activities of the vaccine was seen during the incubation but the patterns of the reversion were quite different each other as seen in Fig. 3. Although a control test on reversion of the EHS activity of acellular pertussis vaccine was introduced into the Minimum Requirements in 1991, it was revealed in the present study that the maximal activity of the LHS after reversion has been remained uncontrolled.

An attempt was made to explore further the difference between the two HS activities by comparing their susceptibilities to anti-PT serum. The reference vaccine was used in the neutralization experiment instead of an acellular vaccine because it lacked the EHS activity enough for the experiment. The hypersensitivity to histamine of mice induced by the reference vaccine did not show a clear peak on the fourth day of sensitization but showed persisting strong sensitization up to the 10th day in the present study unlike the results in previous reports (10,11) in which clear peaks of histamine sensitization were noted on the fourth and 12th days of sensitizing mice with a whole cell and an acellular pertussis vaccine, respectively. The difference in mortalities of mice after histamine-challenge between the present results and the previous reports on respective days, however, was not found statistically significant up to the 14th day of sensitization when tested by Fisher's exact test. Various environmental factors such as conditions of housing and feeding are known to influence the histamine sensitization (11), any of these factors, especially difference in housing condition, might be a cause of the slightly different mortality in the present results from that in the previous reports. The serum treatment might affect expression of HS activity by causing a slightly delayed expression after 10 days of sensitization as seen in Fig. 4. In spite of the obscured peak mortality on the fourth day and the slightly delayed expression after the 10th day, it is assumed evident that the anti-PT horse serum treatment resulted in diminished HS activity in the early stage of sensitization leaving the LHS activity which was reported to be observed later than the fourth day of sensitization (10) unaffected.

These findings in this paper revealed that commercial DTP vaccines actually used in Japan generally possess both the HS activities. It was revealed also that these two HS activities behaved in independent manners in changes of their annual means of commercial DTP vaccines and in their reversion during incubating the vaccine at 37 C.

Our recent study by multivariate analysis showed a possible relation of LHS activity to a serious systemic side effect after the vaccination (unpublished data). It would be assumed, therefore, recommendable to apply separate quality control measures on the EHS and LHS activities of commercial DTP vaccines until their roles in reactogenicity are fully characterized.

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