An international collaborative study of the effect of active pertussis toxin on the modified Kendrick test for acellular pertussis vaccines

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ABSTRACT

Speculation that the Japanese modified intra-cerebral challenge assay, which is used in several countries for control of acellular pertussis vaccines, depends on the presence of small amounts of active pertussis toxin led to an assumption that it may not be appropriate for highly toxoided or genetically detoxified vaccines. Consequently, at the recommendation of a World Health Organisation AD Hoc Working Group on mouse protection models for testing and control of acellular pertussis vaccine, the effect of pertussis toxin on the modified intra-cerebral challenge assay (modified Kendrick, MICA) was evaluated in an international collaborative study. Results of this study showed that for genetically detoxified vaccines both with and without active pertussis toxin the MICA clearly distinguished mice vaccinated with acellular vaccines from unvaccinated mice and gave a significant dose–response relationship. However, vaccine samples containing active pertussis toxin (5 or 50 ng/single human dose) appeared to be more potent than the equivalent sample without active pertussis toxin. Similar results were also given by two respiratory infection models (intranasal and aerosol) included in the study. The results also indicated that the effect of pertussis toxin may vary depending on mouse strain.

1. Introduction

Acellular pertussis vaccines (ACVs) have been shown to be both safe and efficacious in clinical trials in several European countries and Senegal [1–6]. These products are now licensed for routine use in many countries. Although licensed products are expected to be consistent with clinical trial lots, there is no internationally agreed potency assay in general use to assure this. Problems in standardising and controlling ACVs include varied antigenic composition, different detoxification methods, absence of unequivocal correlates of protection and lack of an independently validated and generally accepted animal model [7–9].

Currently, the modified intra-cerebral challenge assay (MICA, modified Kendrick test) is used in Japan, Korea and China and possibly other Asian countries as the potency assay for routine release of acellular pertussis and combination vaccines [10]. For release, vaccines must have potency ≥4 unit (unit as defined by national reference standard)/dose with lower 95% confidence limit ≥2 unit/dose [11,12]. The vaccines which meet release criteria in these countries are known to be clinically effective [13–19]. However, it has been claimed that the MICA may not be appropriate for highly toxoided or genetically detoxified vaccines because it depends on the presence of small amounts of active pertussis toxin (PT) [8,20,21]. At a meeting of representatives of vaccine manufacturers, regulatory authorities and members of the World Health Organisation (WHO) AD Hoc Working Group on mouse protection models for testing and control of ACVs, it was recommended that a collaborative investigation on the effect of PT on MICA should be carried out [8]. It was also noted that other factors might affect the MICA, and that these should be investigated if possible. The National Institute for Biological Standards and Control (NIBSC), UK was designated to co-ordinate this investigation. Accordingly, an international collaborative study was initiated with the following aims:

1) To compare, using the MICA, the protective effect of ACV containing different concentrations of PT.
2) To provide WHO with more information about this model for evaluation of ACVs based on the results of the study, the experience and comments of the participants.

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3) To compare, if possible, the effect of different mouse strains on determination of active PT content using MICA, and if possible to assess the samples using respiratory challenge models.

2. Participants

A total of eleven laboratories, including vaccine manufacturers and national control laboratories, nine of whom performed MICA and two of whom performed respiratory challenge tests (intranasal and aerosol) for the evaluation of ACVs were invited to participate in the study. Results from eight laboratories performing MICA and two laboratories performing respiratory challenge, listed in Table 1, were reported to NIBSC. Throughout this report participants are identified only by a randomly assigned number from 1 to 11. Separate assays have been numbered sequentially within laboratories.

3. Materials

The participating laboratories were asked to evaluate 3 samples of ACV and an aluminium adjuvant control, all of which were coded by letter, together with JNIH-3 (coded D) as working reference preparation. The details of these samples are given in Table 2. Participants were also asked to include their in-house reference (IHR) (if possible).

4. Study design

Participants were asked to carry out MICA [11,12] using their own methodology, reagents and animal strains, including challenge strains and controls used routinely in their laboratory. Each participant was requested to carry out at least two independent assays for each sample and to complete and return to NIBSC a

Table 1
Participating laboratories.

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National Institute of Quality Control in Health (INCQS), Fundacao Oswaldo Cruz, Av. Brasil 4365-Manguinhos, Rio de Janeiro/RJ, CEP 21. 045-900, Brasil
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Table 2
Sample information.

Sample code	Description
A	3-component DTPa vaccine, each 0.5 ml dose contains 5 µg genetically detoxified PT, 2.5 µg FHA, 2.5 µg pertactin, 25 Lf Dip, 10Lf Tet with aluminium hydroxide as adjuvant.
B	Sample A + 5 ng PT/0.5 ml
C	Aluminium hydroxide (contains 0.2% Al(OH)$_3$)
D	JNIH-3, 2-components freeze-dried acellular pertussis vaccine preparation contains 7.5 µg (PN) PT and 7.5 µg (PN) FHA and have a nominal potency of 40 units of pertussis vaccine per ampoule[a].
E	Sample A + 50 ng PT/0.5 ml

[a] Subsequent to this study, JNIH-3 has been established as the First International Standard for Acellular Pertussis Vaccine for use in the MICA and assigned a potency of 34 IU/ampoule [23].

description of their detailed assay procedure together with the raw data for each assay to permit data analysis using as far as possible a common method. If more than one mouse strain was available, participants were requested where possible to repeat the assay to investigate the effect of different mouse strains. Two assays were also carried out using intranasal challenge (INC) [22], and two assays were carried out using aerosol challenge (AC) [23]. Information about assay methods, as received from participants, is given in Table 3.

5. Statistical analysis

Data from each MICA have been analysed as a multiple parallel line assay [24] relating the probits of the proportion of mice surviving to the log of dose of each vaccine preparation using iterative maximum likelihood estimation. Where data permitted, the lethal dose 50% (LD$_{50}$) of the challenge strains was also calculated using probit transformed proportions responding. Validity of individual assays has been assessed in terms of total deviations from the linear parallel line model for the multiple comparisons of preparations. Any assays for which the deviations from either linearity or parallelism were significant ($p < 0.05$) and for which the total deviations from the model were also significant ($p < 0.05$) were taken to be invalid, and estimates from these assays have not been included in the calculated mean estimates.

Data from the AC assays conformed to the conditions for a multiple parallel line assay relating the log of observed colony forming units (CFU) at 7 days to the log of dose of each vaccine preparation. Estimates of potency and weights are shown with estimates of potency for the MICAs.

Data for the INC assays consisted of observed CFU at several time points following administration of the same nominal total dose of each vaccine preparation. Since only a single total dose of each vaccine sample has been tested, any estimation of relative potency would necessarily be conditional on assumptions about the (unknown) nature of the relationship of the response to total dose of vaccine. For these assays, and for the AC assays (using responses for only the larger of the two doses of each vaccine to give comparability with the INC assays), 'potency' has been expressed as ratios of absolute activities, although it has been shown in previous studies that the values of such relative activities are highly dependent on the individual assay. For the AC assays, both relative activities and relative potency estimates have been calculated and can be compared.

Estimates of potency have been assessed for homogeneity using a χ^2 test. Homogeneous estimates have been combined as weighted geometric means, using as weight the reciprocal of the asymptotic variance of the log potency. The reported weights reflect the