

The effect of the addition of *Bordetella parapertussis* suspensions on the protective power of the pertussis component of diphtheria-tetanus-pertussis vaccines

By N. KÖHLER-KUBELKA

Institute of Immunology, Zagreb, Yugoslavia

(Received 10 January 1967)

INTRODUCTION

Previous experiments (Köhler-Kubelka, 1962) have shown that the addition of a parapertussis component to diphtheria-tetanus-pertussis vaccine lowered to some extent the agglutinogenic potency of the pertussis component. In the present work we have continued our investigations into this problem in order to find out to what extent the addition of the parapertussis organisms reduces the agglutinogenic potency of the pertussis component, and to find out whether the addition affects the protective power of pertussis vaccines as measured by active immunization tests in mice.

METHODS

Three batches of diphtheria-tetanus-pertussis (DTP) vaccine, numbers 26, 27 and 28, were prepared containing in each 0.5 ml. (human) dose: 20 Lf diphtheria toxoid, 10 B.U. tetanus toxoid, 12×10^9 organisms of *Bordetella pertussis*, 2 mg. AlPO_4 and merthiolate to a final concentration of 1/10,000. Six samples of vaccine were prepared from each of the three batches and to five of them parapertussis suspension was added to give final concentrations per 0.5 ml. of 0.25×10^9 , 0.5×10^9 , 1×10^9 , 2×10^9 , and 3×10^9 . The sixth sample of each batch was left free from parapertussis component.

The parapertussis component was prepared by mixing equal parts of *Bordetella parapertussis* strains no. 37/56, 16/58, 32/59 and 50/59. The vaccine was heated at 56° C. for 30 min. and merthiolate to a final concentration of 1/10,000 was added.

The 18 vaccine samples under test were tested for agglutinin production by the method of Evans & Perkins (1953). Batches of 15 female mice, each weighing 14 g., were inoculated with 0.5 ml. of each of the 18 samples and bled 24 days later. The agglutinin titres of each mouse for pertussis and for parapertussis were determined by the author's own modification (Köhler-Kubelka, 1957) of Detlor's (1951) micro-agglutination test. The results are expressed in Table 1 as the geometric mean titre produced in each group of mice.

The 18 vaccine samples were also tested by the active mouse protection test according to the Requirements for Pertussis Vaccine (1964). The ED₅₀ values (the calculated amount of vaccine that will protect 50% of the mice) of all samples were calculated by the probit method and a comparison made between the test and the control vaccine samples.

RESULTS

The results are presented in Table 1.

The average agglutinin titres for parapertussis ranged from 1/932 to 1/20,945. The mice that received the higher doses of parapertussis gave the higher agglutinin titres against parapertussis and vice versa. The mice that received the control vaccines without the parapertussis component had no agglutinin titres to parapertussis.

Table 1. *Agglutinin titres and protective properties of various pertussis-parapertussis components of DTP vaccines*

Vaccine batch no.	Pertussis (millions per mouse dose)*	Parapertussis (millions per mouse dose)	Agglutinin titre†		ED 50‡ (millions)
			Pertussis	Parapertussis	
26-1	2,000	500	171	11,578	57.68
26-2	2,000	333	106	10,240	74.28
26-3	2,000	166	335	2,793	50.08
26-4	2,000	83	673	2,360	30.44
26-5	2,000	41	683	932	48.88
26-6	2,000	0	705	0	48.16
27-1	2,000	500	256	19,588	89.56
27-2	2,000	333	132	20,940	121.92
27-3	2,000	166	150	5,184	94.68
27-4	2,000	83	250	4,456	94.16
27-5	2,000	41	336	1,750	102.04
27-6	2,000	0	414	0	81.28
28-1	2,000	500	34	19,456	332.16
28-2	2,000	333	100	20,945	197.24
28-3	2,000	166	150	7,240	311.48
28-4	2,000	83	140	5,497	131.08
28-5	2,000	41	120	2,078	237.08
28-6	2,000	0	198	0	102.20

* Composition of pertussis-parapertussis component: mouse dose one-sixth of human dose.

† Reciprocal of average agglutinin titre in each batch of mice.

‡ 26-1-26-6: $\chi^2h = 3.5671 < \chi^2hk$ 27-1-27-6: $\chi^2h = 6.2093 < \chi^2hk$
 $\chi^2p = 2.5214 < \chi^2hp$ $\chi^2p = 9.4795 < \chi^2hp$
 28-1-28-6: $\chi^2h = 2.0124 < \chi^2hk$
 $\chi^2p = 3.2807 < \chi^2hp$

The average agglutinin titres for pertussis were lower in the mice inoculated with the vaccine containing parapertussis; in general one can say that the fall in pertussis titre was inversely proportional to the rise in parapertussis vaccine dose. The agglutinin titres against parapertussis were several times greater than those against pertussis.

The protective power of pertussis vaccine in mice was expressed in ED 50. The results show no significant difference in the degree of protection conferred by the vaccines with or without the parapertussis component, nor was there any significant difference between the various parapertussis-containing vaccines whether they contained 2% or 25% of the pertussis component. A simple statistical analysis of

the samples showed that all of them satisfy the conditions of homogeneity and parallelism; the deviations from parallelism are not significant nor are the fluctuations in heterogeneity.

DISCUSSION

It can be seen from the results that the addition of a parapertussis component to diphtheria-tetanus-pertussis vaccine reduces the agglutinin titres to the pertussis component, this being particularly evident when the amount of the parapertussis added was 25 % of the pertussis component; with smaller amounts of parapertussis this difference is less striking.

The agglutinin-eliciting property of parapertussis organisms in mice is markedly higher than that of pertussis organisms, as has been reported previously by the author (Köhler-Kubelka, 1962) and was confirmed by Malivanova (1966). In a combined pertussis-parapertussis vaccine the substantially higher agglutinogenic potency of the parapertussis organisms seems to block the immunogenic apparatus of the mice and make it incapable of reacting adequately in producing agglutinin against the pertussis component. This observation led us to investigate whether this inhibitory influence on agglutinogenic potency was also reflected in the protective powers of pertussis vaccines.

As a rule, *Bordetella pertussis* causes a more severe illness of longer duration in children than does *B. parapertussis*, and the main purpose of vaccination is to fight successfully against *B. pertussis* infection. A possible inhibition of the protective power of pertussis component, caused by the addition of parapertussis organisms, would throw doubt on the efficacy of a combined diphtheria-tetanus-pertussis-parapertussis vaccine or would bring about the necessity of increasing the number of pertussis organisms or reducing the number of parapertussis ones. The results of these tests show that no significant reduction in the level of protection conferred by the pertussis component was found when the added parapertussis component was between 2 and 25 % of the pertussis. Our routine vaccine (DTPP) contains in a 0.5 ml. dose 12×10^9 pertussis organisms, 1×10^9 parapertussis organisms, with diphtheria and tetanus toxoids; this combination (26-3, 27-3, 28-3) produced in mice, we considered, a sufficiently high agglutinin titre for parapertussis while the mouse protective power of the pertussis component remained unchanged within the accuracy of the mouse assay.

SUMMARY

The addition of a parapertussis component to diphtheria-tetanus-pertussis vaccine reduces the agglutinogenic potency of the pertussis component in mice. The loss is evident when the amount of the parapertussis component added is 25 % of that of the pertussis component, while the influence of relatively lower amounts of parapertussis organisms is less striking.

The parapertussis component in these vaccines, amounting to 2-25 % of the pertussis component, does not affect the protective power of the pertussis component in mice.

I thank Prof. Ikić, Director of the Institute of Immunology, Zagreb, for his help, Dr Lj. Mravunac for her advice, and Mrs N. Delimar for the statistical analysis of results.

REFERENCES

- EVANS, D. G. & PERKINS, F. T. (1953). An agglutinin-production test in the study of pertussis vaccines. *J. Path. Bact.* **66**, 479.
- DETLOB, M. (1951). Agglutination of *H. pertussis* using concavity slides. *J. clin. Path.* **4**, 487.
- KÖHLER-KUBELKA, N. (1957). Results of serological examination in children vaccinated with our Di-Te-Per vaccine. *Lijecn. Vijesn.* **79**, 754.
- KÖHLER-KUBELKA, N. (1962). Diphtheria-tetanus-pertussis vaccine combined with *Bordetella parapertussis* vaccine. *J. Hyg., Camb.* **60**, 289.
- MALIVANOVA, O. M. (1966). Determination of the parapertussis vaccine efficacy in experimental conditions. *Zh. Mikrobiol. Épidem. Immunobiol.* **43**, no. 2, 10.
- REQUIREMENTS FOR PERTUSSIS VACCINE (1964). *Tech. Rep. Ser. Wld Hlth Org.* no. 274, p. 25.