

Aluminum salts in vaccines—US perspective[☆]

Norman W. Baylor*, William Egan, Paul Richman

Food and Drug Administration, Center for Biologics Evaluation and Research, Office of Vaccines Research and Review, Bethesda, MD, USA

Received 4 June 2001; received in revised form 20 September 2001; accepted 21 September 2001

Abstract

Aluminum in the form of aluminum hydroxide, aluminum phosphate or alum has been commonly used as an adjuvant in many vaccines licensed by the US Food and Drug Administration. Chapter 21 of the US Code of Federal Regulations [610.15(a)] limits the amount of aluminum in biological products, including vaccines, to 0.85 mg/dose. The amount of aluminum in vaccines currently licensed in the US ranges from 0.85–0.125 mg/dose. Clinical studies have demonstrated that aluminum enhances the antigenicity of some vaccines such as diphtheria and tetanus toxoids. Moreover, aluminum-adsorbed diphtheria and tetanus toxoids are distinctly more effective than plain fluid toxoids for primary immunization of children. There is little difference between plain and adsorbed toxoids for booster immunization. Aluminum adjuvants have a demonstrated safety profile of over six decades; however, these adjuvants have been associated with severe local reactions such as erythema, subcutaneous nodules and contact hypersensitivity. Published by Elsevier Science Ltd.

Keywords: Aluminum; Adjuvants; Vaccines; Clinical trials

1. Introduction

One of the earliest uses of aluminum as an adjuvant was reported by Glenny et al. in 1926 [1]. These investigators demonstrated that the addition of potassium alum to diphtheria toxin resulted in a precipitate, and depending on the added amount of potassium alum, the remaining filtrate no longer contained toxin. The precipitated diphtheria toxin when injected into guinea pigs resulted in a higher antigenic response as compared to fluid toxoid.

Aluminum in the form of aluminum hydroxide ($\text{Al}(\text{OH})_3$), aluminum phosphate (AlPO_4) or alum ($\text{KAl}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) continues to be commonly used as an adjuvant in vaccines [2]. Aluminum hydroxide is a crystalline aluminumoxyhydroxide that is positively charged at physiological pH [isoelectric point (pI) = 11]. Aluminum phosphate is an amorphous aluminum hydroxyphosphate which is negatively charged at physiological pH (pI = 5–7). In alum-precipitated vaccines, alum is an aluminum hydroxide that contains some sulfate anions as well as anions that are used in the buffer, often phosphate. The pI depends on the precipitation process and is usually in the range of

0.3–0.6. Two methods have commonly been used to prepare aluminum adjuvanted vaccines [3]. One method involves adding a solution of alum to an antigen to create a precipitate of protein aluminate; these products are designated alum-precipitated vaccines. The second method involves adding an antigen to pre-formed aluminum hydroxide or aluminum phosphate; this results in an aluminum-adsorbed vaccine.

Aluminum compounds are the only adjuvants used in the manufacture of currently licensed vaccines in the United States. Chapter 21 of the US Code of Federal Regulations [610.15(a)] governs the amount of aluminum permitted in the recommended single human dose of a product. The amount of aluminum is limited to no >0.85 mg/dose if the level is assayed, or 1.14 mg if determined by calculation on the basis of the amount of aluminum compound added. The regulations were amended in 1981 to increase the permissible level of aluminum to 1.25 mg in biological products to make the regulations consistent with the World Health Organization standards per single human dose of a product. If aluminum compounds other than alum are used, the total amount of alum should not be more than the equivalent permitted as potassium alum, i.e. 15 mg [4]. The amount of 15 mg of alum or 0.85 mg aluminum per dose was selected empirically from data that demonstrated that this amount of aluminum enhanced the antigenicity and effectiveness of the vaccine (Joan May, FDA/CBER, personal communication).

The immunogenicity of antigens adsorbed onto aluminum adjuvants depends on several factors; however, the most

[☆] The views in this article are those of the authors and are not intended to represent those of the Food and Drug Administration or the Public Health Service.

* Corresponding author. Present address: 1401 Rockville Pike, Rockville, MD 20852, USA. Tel.: +1-301-827-0655; fax: +1-301-827-0448.

E-mail address: baylor@cber.fda.gov (N.W. Baylor).

important is the degree of adsorption of antigen on the adjuvant and the dose of adjuvant. Gupta et al. [2] demonstrated this in mice. The formulation of DT that did not show any adsorption of DT to aluminum phosphate because of a 10-fold excess of phosphate did not elicit antibodies after the first injection, and showed a poor response after the second injection. As far as dose, a small amount of adjuvant may be required for complete adsorption. Even though small doses may completely adsorb the antigens, they may not show an optimal adjuvant effect [5]. Excessive amounts of aluminum compounds may suppress immunity by covering the antigen completely with mineral compounds or the aluminum compounds may be cytotoxic to macrophages [2]. Aluminum hydroxide has been demonstrated to have a more potent adjuvant effect than aluminum phosphate which may be due to its higher adsorption capacity and better adsorption of certain antigens at neutral pH [2].

2. Clinical evaluation

Most of the clinical studies examining the effectiveness of aluminum adjuvanted vaccines were done in the

1930s, 1940s and 1950s, and the results varied depending on the antigens involved. One such study (Table 1) compared the antigenic responses of fluid diphtheria toxoid with alum-precipitated toxoid in children of various ages [6]. The results of this study showed that one dose of alum-precipitated toxoid were inferior to three doses of fluid toxoid; however, two doses of alum-precipitated toxoid were far superior to three doses of plain toxoid. Moreover, these investigators found that two doses of alum-precipitated toxoid stimulated a longer lasting immune response as noted by a high proportion (86%) of children possessing detectable antitoxin 3 years after immunization. Another study (Table 2) compared the immune responses to plain versus alum-precipitated diphtheria toxoid in infants 6–10 days of age [7]. The infants were given either two doses intramuscularly of alum-precipitated diphtheria toxoid 8 weeks apart or three doses of plain toxoid (25 Lf) at monthly intervals. Detectable levels of antitoxin were found in all of the infants who received two doses of alum-precipitated toxoid; however, 6 out of 15 infants receiving plain toxoid did not respond.

Three independent clinical trials of a multivalent vaccine containing diphtheria, tetanus and pertussis toxoids

Table 1
Diphtheria toxoid, fluid vs. alum precipitated^a

| Immunization procedure (20Lf toxoid per dose) | Percentage of children showing detectable antitoxin (0.01 U/ml) | |
|---|---|-------------------------------|
| | 4 months after first injection | 3 years after first injection |
| Fluid, one dose | 8 | 4 |
| Fluid, two doses | 29 | 19 |
| Fluid, three doses | 67 | 56 |
| Alum ppt., one dose | 56 | 26 |
| Alum ppt., two doses | 96 | 86 |

^a From [6]. Copyright 1942 by the American Public Health Association.

Table 2
Diphtheria toxoid: fluid vs. alum precipitated^a

| Age of children at time of first injection | Injections given | No. of infants with detectable antitoxin/no. injected |
|--|-----------------------------|---|
| 6–10 days | Plain toxoid, 3 × 25 Lf | 9/15 |
| 6–10 days | Alum ppt. toxoid, 2 × 25 Lf | 23/23 |
| ≥7 months | Plain toxoid, 3 × 25 Lf | 6/6 |
| ≥6 weeks | Alum ppt. toxoid, 2 × 25 Lf | 43/43 |

^a From [7].

Table 3
DTP trial, antitoxin responses^a

| Group | Geometric mean antitoxin U/ml | | | | | |
|---|-------------------------------|-----------|-----------------------|----------|-----------|-----------------------|
| | Diphtheria | | | Tetanus | | |
| | 6 months | 12 months | 3 months post-booster | 6 months | 12 months | 3 months post-booster |
| Al(OH) ₃ vaccine (61 infants) injected at 1, 6, and 14 weeks | 0.4 | 0.07 | 1.5 | 3.2 | 1.0 | 8.2 |
| Plain vaccine (77 infants) injected at 6, 12 and 18 weeks | 0.2 | 0.1 | 1.2 | 4.2 | 1.9 | 10.1 |

^a From [8] (Br Med J 1955;2:635–9, reproduced with permission from the BMJ Publishing Group. Copyright 1955 BMJ Publishing Group).

Table 4
DTP trial, antitoxin levels one month after the last of three primary 0.5 ml injections^a

| DTP preparation (concentration 3×10^9 pertussis/ml) | No. of children | Median antitoxin titer | |
|--|-----------------|------------------------|---------|
| | | Diphtheria | Tetanus |
| Plain: D 50Lf T 20Lf | 19 | 0.128 | 0.256 |
| 2.5 mg D 10Lf AlPO ₄ : T 5Lf per ml | 20 | 0.128–0.256 | 0.256 |
| 5.0 mg D 20Lf AlPO ₄ : T 20Lf per ml | 16 | 1.024 | 1.024 |

^a From [9] (*J Am Med Assoc* 1956;160:108–13. Copyright 1956, American Medical Association).

combined (DTP) with and without aluminum adjuvant gave somewhat mixed results. The earliest study of the three compared DTP adsorbed on aluminum hydroxide to plain vaccine [8]. No significant difference in diphtheria and tetanus geometric mean titers was observed between the groups receiving either the plain or the adjuvanted vaccine (Table 3). However, an increase in seroconversion rates was observed with the adjuvanted vaccine (all 61 children receiving the adjuvanted vaccine had diphtheria titers >0.05 whereas 14/77 children in the plain vaccine group were below this value). Four children had no detectable titers.

The second trial involved children receiving DTP adsorbed on aluminum phosphate adjuvant [9]. This study used decreasing amounts of toxoid with different amounts of adjuvant. A significant increase in diphtheria and tetanus antitoxin titers was achieved, especially with increased amounts of aluminum phosphate adjuvant (Table 4). The third trial, in infants, comparing plain DTP with DTP adsorbed on aluminum phosphate showed no significant difference [10]. None of the aluminum adjuvants enhanced the protective effectiveness of pertussis toxoid in either trial because pertussis toxoid can behave as an adjuvant [11,12].

Variations in the results observed in different studies may be due to differences in antigenic dose, stability of the aluminum–antigen complexes, intrinsic adjuvant effect of the pertussis component on the antitoxin response, and in infants, levels of circulating maternal antitoxin [7,13]. However, the consensus of the early studies suggests that aluminum-adsorbed toxoid vaccines are distinctly more effective than plain toxoids for the primary immunization of children, whereas, for booster immunization little difference is observed between plain and adsorbed toxoids.

3. Safety

There have been differences in opinion on using aluminum adjuvants in vaccines for human use. For example, the British Ministry of Health recommended aluminum-free vaccines in 1957, whereas, in 1964 the Committee on Control of Infectious Diseases of the American Academy of Pediatrics advised the use of alum-precipitated DTP or vaccine adsorbed on aluminum hydroxide or aluminum phosphate [11]. Nevertheless, in general, vaccines using aluminum adjuvants have a demonstrated safety profile of more than six

decades. Aluminum-containing vaccines have been associated with severe local reaction such as erythema, subcutaneous nodules, contact hypersensitivity and granulomatous inflammation [2]. Some studies with aluminum-adsorbed DTP vaccine have reported fewer reactions than unadsorbed vaccine [14,15]. Aluminum hydroxide has been reported to attract eosinophils to the injection site [16], and may increase the levels of antigen-specific and total IgE antibodies that may promote IgE-mediated allergic reactions [17]. On the other hand, aluminum adjuvants have been used for years for hyposensitization of allergic patients without adverse results [2]. There have also been reports, especially in patients with impaired renal function, of systemic accumulation of aluminum, which has been associated with nervous disorders and bone disease [18]. Nonetheless, aluminum intake from vaccines is far less than that received from the diet or medications such as antacids.

4. Aluminum content in currently licensed vaccines

As previously stated, the US FDA (21 CFR 610.15(a)) allows no >0.85 mg/dose of aluminum in vaccines. The amount of aluminum in currently licensed vaccines is listed in Table 5, and ranges from 0.85 mg of aluminum per dose for Lederle's DTP-Haemophilus influenza type b conjugate combined vaccine to 0.125 mg of aluminum per dose for Lederle's recently licensed pneumococcal conjugate vaccine. The currently licensed vaccines use alum, aluminum hydroxide, aluminum phosphate, or a combination of aluminum hydroxide and aluminum phosphate. Those licensed vaccines not listed in Table 5 such as the live viral vaccines, inactivated polio vaccines; influenza; rabies (Aventis Pasteur, and Chiron Behring), yellow fever, Japanese encephalitis, adenovirus, pneumococcal polysaccharide, typhoid, plague, cholera, BCG, meningococcal, and all Haemophilus influenza type b conjugate vaccines (except Merck's) do not contain aluminum adjuvants.

Table 6 contains the calculated amounts of aluminum a child or adult may receive from vaccinations. The total amount of aluminum received from vaccines will vary depending on which brand of vaccine is given. A 1-year-old who receives a complete series of recommended vaccines may receive a minimum of 1.6 mg of aluminum or a maximum of 4.1 mg of aluminum from these vaccines. A 5-year-old would be exposed to a similar amount of

Table 5
Aluminum content of licensed vaccines^a

| Vaccine | Trade name | Manufacturer | Al per dose (µg) | Chemical form of Al | No. of doses in series (mg) | Total Al for series | Comments |
|--|-----------------|------------------|------------------|-------------------------|-----------------------------|---------------------|--|
| Childhood vaccines | | | | | | | |
| DTaP | Infanrix | SKB | ≤625 | Hydroxide | 5 | 3.1 | |
| | Certiva | NAVA | 500 | Hydroxide | 5 | 2.5 | |
| | Acelimune | Lederle | 230 | Hydroxide/ Phosphate | 5 | 1.2 | |
| | Tripedia | Avent. Past Inc. | ≤170 | Alum | 5 | 0.85 | |
| DTP | – | BioPort | ≤600 | Phosphate | 5 | 3.0 | |
| | – | Av. Past Inc. | ≤170 | Alum | 5 | 0.85 | |
| Hib conjugate | Liq. Pedvax Hib | Merck | 225 | Hydroxide | 3 | 0.68 | |
| Pneumo conjugate | Prevenar | Lederle | 125 | Phosphate | 3 | 0.38 | |
| DTP–Hib | Tetramune | Lederle | ≤850 | Hydroxide | 4 | 3.4 | |
| Hep B–Hib | Comvax | Merck | 225 | Hydroxide | 3 | 0.68 | |
| Hep B | Recombivax B | Merck | 225 | Hydroxide | 3 | 0.68 | |
| | Engerix B | SKB | 250 | Hydroxide | 3 | 0.75 | Additional boosters may be given |
| DT, adsorbed | – | Avent. Past Inc. | ≤170 | Alum | 5 | 0.85 | |
| | – | MPHBL | 450 | Phosphate | 5 | 2.3 | |
| | – | Bioport | ≤600 | Phosphate | 5 | 3.0 | |
| | – | Lederle | ≤800 | Phosphate | 5 | 4.0 | |
| | – | Wyeth | ≤850 | Phosphate | 5 | 4.3 | |
| Adult vaccines (some may also be indicated for younger age groups) | | | | | | | |
| T, adsorbed | – | Lederle | ≤850 | Phosphate | 6 | 5.1 | For booster every 10 years from age 10–60 |
| | – | MPHBL | 450 | Phosphate | 6 | 2.7 | |
| | – | Wyeth | ≤850 | Phosphate | 6 | 5.1 | |
| | – | SSVI | ≤850 | Phosphate | 6 | 5.1 | |
| | – | Avent. Past Inc. | ≤250 | Alum | 6 | 1.5 | |
| Td, adsorbed | – | Lederle | <800 | Phosphate | 6 | 4.8 | For booster every 10 years from age 10–60 |
| | – | Wyeth | ≤850 | Phosphate | 6 | 5.1 | |
| | – | MPHBL | 450 | Phosphate | 6 | 2.7 | |
| | – | Avent. Past Inc. | ≤280 | Alum | 6 | 1.7 | |
| Hep A | Havrix | SKB | 250 | Hydroxide | 2 | 0.5 | Adults; ped/teens get half dose |
| | VAQTA | Merck | 450 | Hydroxide | 2 | 0.9 | Adults |
| | VAQTA | Merck | 225 | Hydroxide | 2 | 0.45 | 2–17 years |
| Lyme | Lymerix | SKB | 500 | Hydroxide | 3 | 1.5 | Booster schedule not established; not currently indicated for children |
| Anthrax | Lymerix | BioPort | ≤830 | Hydroxide | 6 | 5.0 | Yearly booster can be given |
| Rabies | RabAvert | BioPort | 442 | Phosphate | 5 | 2.2 | Post-exposure |
| | RabAvert | BioPort | 442 | Phosphate | 3 | 1.3 | Pre-exposure; booster 2× per year can be given |

^a For values designated as < or ≤, the actual aluminum content may be significantly less.

aluminum, 1.9–4.9 mg, if the recommended vaccines for this age were received. The amount of aluminum an adult would receive from vaccines varies greatly depending on the number of vaccines given. The Advisory Committee on Immunization Practices recommends a Td booster every 10 years and recommends influenza vaccine for those over 60 years of age; most of the other vaccines listed would not be routinely given to all adults.

5. Summary

Although there are only a few clinical trials in which a given batch of vaccine, with and without adjuvant, has been tested in comparable populations, aluminum adjuvants have been used in vaccines for many decades, and have been proven to be safe. A review of the literature on aluminum-adsorbed or alum-precipitated vaccines suggests

Table 6
Estimated Al loading from vaccination

| Age | Vaccine | Doses in series | Al/series (mg) | | Total Al (mg) | |
|--|-------------------------------------|-----------------|-------------------|------|---------------|------|
| | | | Min | Max | Min | Max |
| 1 | DTaP | 3 | 0.51 | 1.88 | 1.6 | 3.5 |
| | Hib conjugate ^a | 2 | 0 | 0.45 | 1.6 | 3.5 |
| | Pneumo conjugate | 3 | 0.38 | 0.38 | 1.6 | 3.5 |
| | Hep B | 3 | 0.68 | 0.75 | 1.6 | 3.5 |
| | Tetramune | 3 | 2.55 | 2.55 | 3.6 | 3.7 |
| | Pneumo conjugate | 3 | 0.38 | 0.38 | 3.6 | 3.7 |
| | Hep B | 3 | 0.68 | 0.75 | 3.6 | 3.7 |
| | DT adsorbed | 3 | 0.51 | 2.55 | 3.6 | 3.7 |
| | Hib conjugate | 2 | 0 | 0.45 | 1.6 | 4.1 |
| | Pneumo conjugate | 3 | 0.38 | 0.38 | 1.6 | 4.1 |
| 5 | Hep B | 3 | 0.68 | 0.75 | 1.6 | 4.1 |
| | Cumulative | – | – | – | 1.9 | 4.9 |
| | DTaP | 5 | 0.85 ^c | 3.13 | 1.9 | 4.1 |
| | Hib conjugate | 3 | 0 | 0.68 | 1.9 | 4.9 |
| | Pneumo conjugate | 3 | 0.38 | 0.38 | 1.9 | 4.9 |
| 60 | Hep B | 3 | 0.68 | 0.75 | 1.9 | 4.9 |
| | Above childhood | – | 1.91 | 4.94 | 10.6 | 4.9 |
| | Td (boost every 10 years) | 6 | 1.68 | 5.10 | 10.6 | 4.9 |
| | Hep A (1 series) | 2 | 0.50 | 0.90 | 10.6 | 19.6 |
| | Lyme (1 series) | 3 | 1.50 | 1.50 | 10.6 | 19.6 |
| | Anthrax (1 series) | 6 | 5.00 | 5.00 | 10.6 | 19.6 |
| 60 (high risk, e.g. lab worker) ^d | Rabies (post-exposure) ^b | 5 | 0 | 2.2 | 10.6 | 19.6 |
| | Above vaccines | – | 10.6 | 19.6 | 31.4 | 19.6 |
| | Anthrax (once yearly boost) | 25 | 20.8 | 20.8 | 31.4 | 62.5 |
| | Rabies (twice yearly boost) | 50 | 0 | 22.1 | 31.4 | 62.5 |

^a Only one of four licensed Hib conjugates contains aluminum.

^b Only one of four licensed rabies vaccines contains aluminum.

^c The schedule with acellular pertussis used.

^d Assuming 25 years of booster vaccinations with rabies and/or anthrax.

that adjuvants may not be necessary in booster immunizations. Further, not all vaccines have an enhanced antigenicity when adsorbed to aluminum, e.g. pertussis [12] and typhoid fever [19]. To produce separate formulations for the primary and booster series, with and without adjuvant respectively, would be impractical from a manufacturing point of view. Other limitations of aluminum adjuvants include local reactions, production of IgE antibodies, and the inability to elicit cell-mediated immunity. There are alternative adjuvants under study; however, to replace aluminum adjuvants in currently licensed vaccines would encompass a new product requiring safety and immunogenicity studies. Finally, the enhanced antigenicity of some vaccines may be sacrificed if aluminum-containing adjuvants were to be completely removed from these vaccines.

References

- [1] Glenney AT, Pope CG, Waddington H, Wallace U. XXIII—the antigenic value of toxoid precipitated by potassium alum. *J Pathol Bacteriol* 1926;29:38–9.
- [2] Gupta RK, Rost BE, Relyveld E, Siber GR. Adjuvant properties of aluminum and calcium compounds. In: Powell MF, Newman MJ, editors. *Vaccine design: the subunit and adjuvant approach*. New York: Plenum Press, 1995. p. 229–48.
- [3] Edelman R. Vaccine adjuvants. *Rev Infect Dis* 1980;2(3):370–83.
- [4] Department of Health, Education and Welfare, Public Health Service, National Institutes of Health. Minimum requirements for the amount of aluminum adjuvants in toxoids, vaccines and multiple antigens 1953.
- [5] Jensen OM, Koch C. On the effect of Al(OH)₃ as an immunological adjuvant. *Acta Pathol Microbiol Immunol Scand* 1988;96:257–64.
- [6] Volk VK, Bunney WE. Diphtheria immunization with fluid toxoid and alum-precipitated toxoid. *Am J Public Health* 1942;32:690–9.
- [7] Barr M, Glenney AT, Hignett S, Randall KJ, Thomson A. Antigenic efficiency of fluid and precipitated diphtheria prophylactics in very young babies and lambs. *Lancet* 1952;2:803–5.
- [8] Barr M, Glenney AT, Butler NR. Immunization of babies with diphtheria–tetanus–pertussis prophylactic. *Br Med J* 1955;2:635–9.
- [9] Greenberg L, Benoit R. Control of potency and the dosage of diphtheria and tetanus toxoids. *J Am Med Assoc* 1956;160:108–13.
- [10] Feldman GV. Pertussis antibody response after triple antigen. *Arch Dis Childhood* 1957;32:111–3.
- [11] Wardlaw AC, Aprile MA. Field trials of aluminum adjuvant vaccines and toxoid: a review. In: Regamey RH, editors. *Proceedings of the International Symposium on Adjuvants of Immunity*. Basel, Switzerland: Karger, 1967. p. 257–66.
- [12] Butler NR, Wilson BDR, Benson PF, Dudgeon JA, Ungar J, Beale AJ. Response of infants to pertussis vaccine at 1 week and to

- poliomyelitis, diphtheria and tetanus vaccines at 6 weeks. *Lancet* 1962;2:112–4.
- [13] Fleming DS, Greenberg L, Beith EM. The use of combined antigens in the immunization of infants. *Can Med Assoc J* 1948;59:101–5.
- [14] Hilton ML, Wurland WL. Pertussis containing vaccines: the relationship between laboratory toxicity tests and reactions in children. *Symp Ser Immunobiol* 1970;13:150–6.
- [15] Cameron J. The potency of whooping cough (pertussis) vaccines in Canada. *J Biol Stand* 1980;8:297–302.
- [16] Walls RS. Eosinophil response to alum adjuvants: involvement of T cells in nonantigen-dependent mechanisms. *Proc Soc Exp Biol Med* 1977;156:431–5.
- [17] Nagel J, Svec D, Water T, Fireman P. IgE synthesis in man. Part I. Development of specific IgE antibodies after immunization with tetanus–diphtheria (TD) toxoids. *J Immunol* 1977;118:334–41.
- [18] Food and Drug Administration. Summary Minutes—Allergenic Products Advisory Committee and Report on Safety Considerations for the Aluminum Component of Alum-precipitated Allergenic Extracts, Office of Biologics Research and Review, Biologics Information Staff. Bethesda: FDA, 1987 (NFN-20).
- [19] Cvjetanovic B, Uemura K. The present status of field and laboratory studies of typhoid and paratyphoid vaccines with special reference to studies sponsored by the World Health Organization. *Bull WHO* 1965;32:29–36.