MANUFACTURING CHALLENGES SUPPLEMENT ARTICLE

Manufacturing Issues Related to Combining Different Antigens: An Industry Perspective

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Despite the growing demand for combination vaccines, many challenges have been encountered in developing them. It is difficult to predict the physical compatibility and stability of antigens in combination, because these characteristics are highly dependent on vaccine excipients. Clinical evaluation of potential modifications of efficacy of antigens in combination may be alleviated by use of appropriate animal models. Manufacturing issues, such as batch-release testing, storage of intermediate products, and the shift to preservative-free products, are of particular concern because they have the potential to affect the supply chain. Managing changes in the manufacture of one antigen that is a component of several different combination vaccines is also difficult. However, most potential issues can be resolved through the simplification of regulatory processes and harmonization of requirements, such as the acceptance of comparability protocols and antigen master files. Continued collaboration between industry and authorities is necessary to develop effective means of handling all submissions pertaining to combination vaccines.

There is a global consensus that combination vaccines are greatly needed, especially in view of the changes in vaccination schedules over the past 10 years. To comply with the schedule currently recommended in the United States, an infant would now receive up to 5 injections at one visit: combined diphtheria–tetanus–acellular pertussis vaccine (DTaP), Haemophilus influenzae type b vaccine (Hib), inactivated polio vaccine (IPV), hepatitis B vaccine (Hep B) and conjugate pneumococcal vaccine.

Contrary to initial expectations, the development of combined vaccines has not been straightforward. The challenges facing the pharmaceutical industry in the development of combined vaccines are multiple; this article will examine some of the more pressing technical and manufacturing issues currently being confronted.

FORMULATING COMBINATION VACCINES

The considerable experience of manufacturers over the last decade has demonstrated that formulation of combined vaccines is much more complex than the simple mixing of a limitless number of antigens. Physical compatibility and stability of the individual antigens in combination is highly dependent on vaccine excipients. The type of adjuvant and buffer used, the presence or absence of preservatives, the pH, and tonicity all play an important role in determining the final formulation.

The importance of the choice of adjuvant was made evident during the development of the combined diphtheria–tetanus–whole cell pertussis–Hep B vaccine at GlaxoSmithKline Biologicals (GSK) [1]. It was only after evaluation of 5 different formulations, which differed in the adsorption process and adjuvant used, that the problem of suboptimal immunogenicity of the Hep B component was resolved. The deleterious effect of the preservative thimerosal on the stability of IPV antigens is well known as another difficulty in the manufacture of combinations containing these components [2].
Unfortunately, the potency tests that are routinely used before release of monovalent vaccines often do not predict the occurrence of immune interference with combination vaccines when they are used in humans. Mouse potency tests, for example, do not detect any loss of potency of Hib when combined with DTaP vaccines, whereas a clear interference has been repeatedly observed in clinical trials [3]. Conversely, the enhanced tetanus potency observed in mice who are administered DTaP combined with tetanus-conjugate Hib vaccine is absent in infants.

One of the biggest challenges in the development of combination vaccines is demonstrating that the combination is as efficacious against disease in humans as are its component antigens. This is especially true for vaccines containing pertussis components: antibody levels known to correlate with protection against clinical pertussis have yet to be formally defined. Today, for ethical reasons, a placebo-controlled efficacy trial would no longer be acceptable, given both the high rates of efficacy of the currently licensed acellular pertussis (aP) combinations and the high pertussis vaccine coverage. The problem is exacerbated when in vivo assays are used to evaluate potency. Thus, for batch release. It is evident that this variability is the greatest of all lots produced.

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*a Calculated by the binomial distribution formula.

Of particular concern to industry are the manufacturing issues that affect the supply chain. Disruption of the supply chain may ultimately compromise the continuity of vaccine delivery.

**Batch release testing.** The effects of the development of combined vaccines on routine batch release testing, and especially the risk of having to repeat a potency test due to a nonvalid test result, can easily be estimated by use of a simple mathematical model (table 1). Let us suppose that the release test for each antigen has a theoretical random error of 5% for test failure, which is independent of the actual potency of the antigen in the vaccine. In this instance, 5% of all lots of monovalent vaccine will require repeat testing, and 95% of all lots will pass. A vaccine with 5 valences will, on average, require repeat testing of at least 1 antigen for 23% of all lots produced, whereas a 9-valent vaccine will require repeat testing for 40% of all lots produced.

This simple model graphically illustrates why industry must aim toward the reduction of variability in the test methods used for batch release. It is evident that this variability is the greatest when in vivo assays are used to evaluate potency. Thus, for scientific reasons, a move toward in vitro tests for batch release is justified. A revision of the required tests for batch release is also warranted on ethical grounds. Release testing for 1 lot of tricomponent acellular pertussis vaccine with use of a 50% effective dose range assay requires 140 mice; for a 1-dose assay, testing requires 30 mice. For release testing of diphtheria or tetanus components in accordance with the World Health Organization–European Pharmacopoeia requirements, 90 animals are needed; for testing in accordance with the US Pharmacopoeia guidelines, only 8 animals are needed. A reduction in the number of animals used could therefore be realized through the harmonization of batch release requirements.

Ideally, potency tests should predict efficacy in humans. We
know, unfortunately, that this is often not the case. However, in our view, the most important aim of a potency test is that it adequately monitor the consistency of production. The tests used should demonstrate that production lots are not significantly different from lots evaluated during clinical development, especially those lots that have been demonstrated to be protective in efficacy trials.

As to the choice of reference vaccine used in potency tests for combination vaccines, we feel that both homologous and heterologous standards can be used, provided that their benchmarks are set correctly during the clinical development of the combination vaccine. Indeed, the use of heterologous standards would no doubt simplify testing of vaccine lots by national authorities.

**Storage of intermediate products.** To ensure optimal flexibility, it is important that the bulk antigens used in the final formulation, as well as any intermediate products, can be stored for definite periods before processing. Classically, the validation of storage periods of bulk antigens and intermediate products has been based on the use of these products to formulate lots, which were then tested by use of routine release tests. These lots were followed up for stability, similar to other lots made with “fresh” bulk antigens.

With combination vaccines, multiple bulk products are used in the final formulation process. For most of these antigens, extensive stability data exists for other, less complex formulations. These data should be taken into account for the validation of storage periods of bulk antigens and intermediate products for the larger combination, provided that the compatibility between antigens has been established and that the bulk antigens or intermediate products are identical for the 2 combinations. In practice, this would imply that the registration dossier should contain quality-control results generated at the release of lots that used “fresh” and “aged” bulk products, and that follow-up stability data should be submitted as a postlicensing commitment.

**Managing manufacturing changes.** Manufacturing changes are an unavoidable part of the production process. They may be initiated either by the manufacturer (e.g., following scale-up of production of ≥1 antigen, or process improvement to increase yields) or by regulatory authorities. From the perspective of industry, these latter changes are largely unplanned (examples are removal of thimerosal; a move away from use of raw materials from bovine or other animal origin; or a move toward removal of human albumin from the final product).

Unfortunately, process changes involving 1 antigen have a regulatory impact on all combinations in which this antigen is used. For example, changes in the processing of GSK’s pertussis toxoid would affect the 6 licensed combination vaccines in Europe of which it is a component. It is clear that the regulatory impact of a change in a component such as pertussis toxoid, and the amount of administrative work that would be needed to comply with current regulatory requirements needed to implement changes to 6 licensed products, is enormous.

Vaccine manufacturers therefore need to be proactive; they need to try to foresee eventual production changes, and they need to be selective in the changes that they eventually will implement. From the regulatory perspective, several proposals can be made.

1. **Acceptance of the comparability protocol.** The comparability protocol is already being used in the United States and is currently under discussion in many European countries. Before actual execution of the manufacturing change, the sponsor would submit a detailed validation plan of the changes that will be introduced. Agreement on the plan is reached between the manufacturer and the relevant authorities, which allows the manufacturer to ensure that these changes are acceptable before they are implemented. Once validation data are available, they would be submitted to the authorities, and if all results are within the ranges specified in the original plan, rapid review will follow.

   In the United States, approval is usually granted under the “changes to be effective within 30 days” rule. As vaccine manufacturers, we would hope that the procedures of the Center for Biologics Evaluation and Research change such that comparability protocols can be submitted as an integral part of the initial license application submission, so that a significant amount of time can be gained between approval of the product and implementation of process changes.

2. **Use of antigen drug master files.** A second tool that would help to reduce regulatory complexity is the antigen drug master file. A master file would be created for each antigen, up to the stage of purified preadsorbed bulk. Any manufacturing change occurring during the life cycle of the antigen would be submitted as amendment to this file. The master file would then be cross-referenced to the different approved products that contain this antigen.

3. **Simplification of stability protocols.** The potential impact of manufacturing changes on the stability of the bulk antigen and its combinations needs to be discussed with regulatory authorities. As manufacturers, it is our strong belief that if additional stability data need to be generated, they should be obtained by use of a simplified schedule (i.e., not that recommended by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use), applying a bracketing principle across the different combinations.

4. **Acceptance of technical bridging.** Progress in technology has led to a shift from vaccines that were poorly identified mixtures to those containing specified quantities of purified, well-characterized antigens. In parallel with more rigorous characterization of vaccine components, technical bridging between monocOMPONENT vaccines and combination vaccines...
should become more readily acceptable, with substantial clinical data considered unnecessary for all combinations containing this component.

**SHIFT TO PRESERVATIVE-FREE PRODUCTS**

Because most vaccines contain alum, they cannot be sterilized by filtration at the final bulk stage. Furthermore, as most antigens are preadsorbed, all operations that involve formulation, filling, and eventually freeze-drying need to be performed in aseptic conditions.

The recent move toward preservative-free products carries with it the need to further tighten the validation criteria for aseptic operations. To cope with this challenge, most manufacturers are moving toward the introduction of isolator technology for the filling lines. With this technology, filling lines are physically completely isolated from their environment. This offers an advantage over current technology. However, although such isolated filling lines minimize the likelihood of contamination due to the presence or intervention of personnel, a 100% guarantee that contamination will never occur still cannot be provided.

For combined vaccines, the number of aseptic operations executed during the final blending and formulation far exceeds that of monovalent vaccines. For a 9-valent combination, the number of aseptic operations is $\geq 100$, whereas for a monovalent vaccine this figure is $\sim 20$. To further reduce the risk of contamination during these operations, closed systems for formulation are under development.

Industry is highly committed to the development of preservative-free products in the long term. However, in view of the complexity of the formulation process, this development needs to be progressive and adequately planned. Considerable investments will need to be made in new filling and formulation technologies, and experience in this field will be accumulated gradually. Close interaction with regulatory authorities is needed for agreement, at an early stage, on the characteristics of the equipment to be used, as well as agreement on validation plans. Because of the number of operations implicated in formulation, it is evident that monovalent vaccines will be the first products to be preservative-free, with combination vaccines following at a later stage. Meanwhile, mercury-free preservatives are valid alternatives in instances where preservatives are still in use or required. This is especially the case in situations where multiple-dose presentations are preferred.

**EXPERIENCE WITH COMBINATION VACCINES IN EUROPE**

Combination vaccines, including combinations manufactured by GSK, are widely used in Europe for the primary vaccination of infants. These products include diphtheria and tetanus toxoid and whole-cell pertussis (DTwP) combinations with Hib and Hep B; DTaP combinations with IPV, Hep B, or both; DTaP combinations with Hep B and IPV, with or without Hib; hepatitis A–Hep B vaccines, and Hep B-Hib vaccines. The difference between this situation and the situation in the United States, where fewer combinations are licensed for this indication, can be partially explained by a significant effort to increase vaccine uptake in Europe. This has helped to fuel a shift from DTwP vaccines to the less reactogenic DTaP vaccines, which has occurred in parallel to a desire to decrease the number of injections to be administered.

In agreement with the European authorities, GSK has initiated significant collaborative postmarketing surveillance activities to closely monitor the impact of the introduction of new combined pediatric vaccines on the epidemiology of the diseases targeted and to evaluate vaccine safety after widespread use. Three of the largest programs are outlined below.

**Effectiveness of aP-based vaccines.** As a commitment after licensing of the DTaP-Hep B vaccine, large, multinational surveillance studies were initiated to evaluate long-term protection of vaccine recipients receiving aP-based combination vaccines against clinical pertussis and to monitor the variability of *B. pertussis* strains. Results from the Swedish arm of this study have been published [6]. They show that in the 3 years since the reintroduction of pertussis vaccination (with aP-based vaccines), the reported incidence of pertussis dropped by 80%–90%, to levels observed during the period when whole-cell pertussis vaccines were in wide use. The highest incidence of clinical pertussis was seen in children 5–9 years old, who did not receive pertussis vaccines in infancy.

**Invasive disease due to Hib: effectiveness of Hib combination vaccines.** The combination of Hib vaccines with DTaP-based vaccines has been reported to result in a decrease in the circulating levels of anti–polyribosyl ribitol phosphate after primary vaccination. There is compelling indirect evidence that the combination vaccines will be, nevertheless, highly efficacious, because other indicators of immune response are not altered [7]. In order to confirm that the lower antibody levels would not result in a higher incidence of disease in children, a German surveillance program, conducted under the auspices of the Erhebunseinheit für Pädiatrische Erkrankungen in Deutschland, was amended to include monitoring of invasive Hib disease after licensing of a DTaP-Hib combination in January 1998 [8]. Data from this surveillance program have shown that the incidence of invasive disease has continued to decline in Germany after the introduction of DTaP-Hib and, more recently, DTaP-IPV-Hib vaccine. During the first 12 months of surveillance, the field effectiveness of $\geq 1$ doses of GSK’s Hib combination vaccines was 97%.

**Safety of measles-mumps-rubella vaccines: monitoring of...**

Manufacturing Combination Vaccines • CID 2001:33 (Suppl 4) • S349
events temporally associated with vaccination. A collaborative hospital-linked surveillance program was set up in several regions of the United Kingdom to collect information concerning the occurrence of neurological side effects after vaccination with measles-mumps-rubella vaccines. This study should allow comparison between the trivalent vaccines used in the United Kingdom, 1 of which is manufactured by GSK and was licensed in December 1997.

CONCLUSION

Despite unexpected hurdles, significant progress has been made in the development of new combinations and the improvement of existing vaccines. Many of the challenges that still persist can be reduced through simplification of the regulatory processes and harmonization of regulatory requirements. As vaccine manufacturers, we look forward to continuing discussions on these matters with health authorities, both in the United States and in Europe.

References