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Short paper

Support for the revocation of general safety test regulations in biologics license applications

Dana M. Evans [a], Jennifer M. Thorn [b], Katherine Arch-Douglas [c], Justin B. Sperry [b], Bruce Thompson [b], Heather L. Davis [a], Michael J. McCluskie [a, *]

[a] Pfizer Vaccine Immunotherapeutics, Ottawa Laboratories, Ottawa, ON, Canada
[b] Pfizer Biotherapeutics Pharmaceutical Sciences, St. Louis, MO, USA
[c] Pfizer Worldwide R & D, Peapack, NJ, USA

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ABSTRACT

The United States Food and Drug Administration recently removed the requirement for a General Safety Test (GST) for biologics in the Code of Federal Regulations (21 CFR 610.11). The GST, as well as abnormal toxicity (European Pharmacopeia) and innocuity tests (World Health Organization), were designed to test for extraneous toxic contaminants on each product lot intended for human use. Tests require one-week observations for general health and weight following injection of specified volumes of product batches into guinea pigs and mice. At the volumes specified, dose-related toxicity may result when the product is pharmacologically active in rodents. With vaccines, required doses may be > 3 logs higher than intended human dose on a weight-adjusted basis and if an immune modulatory adjuvant is included, systemic immune hyperactivation may cause toxicity. Herein, using the CpG/alum adjuvant combination we evaluated the different test protocols and showed their unsuitability for this adjuvant combination.

1. Introduction

The United States Food and Drug Administration (FDA) recently amended regulations surrounding lot release of biologics, removing the General Safety Test (GST) requirement [1]. The GST tested for extraneous toxic contaminants on manufactured lots of biologics intended for human use. Similar tests elsewhere are the Abnormal Toxicity Test (ATT) [2] and the innocuity test (IT) [3]. Although previously recommended by the World Health Organization (WHO), these tests have more recently also had their utility questioned such that recent WHO guidelines indicate that the need for these tests should be agreed with the relevant National Regulatory Authority (NRA) and testing may in fact be removed from routine lot release once production consistency has been established and reliable good manufacturing practices (GMP) are in place [4,5].

While some differences exist between the tests, all involve injection of specified volumes of a product batch into guinea pigs and mice followed by a 1-week observation (Table 1). Advances in current manufacturing methods for biologics have reduced the risk of contaminants being carried through the production process. Furthermore, by eliminating the GST requirement, the FDA acknowledged that alternative methods are better suited to evaluate the presence of contaminants in biologics. The value of the GST has been questioned due to its principle deficiencies (specificity, reproducibility, reliability, suitability) [6–8]. In particular, the small number of animals required ($n \leq 5$) makes accurate detection of contamination unlikely [8,9]. In addition, since required volumes in small rodents (e.g., 0.5 mL and 1 full human dose to mice for GST and ATT, respectively) may result in extremely high doses of product on a weight-adjusted basis, observed effects may reflect dose-related toxicity to animals rather than product contamination. These limitations have been partially addressed in Europe by introducing modifications in the ATT for vaccines that may otherwise fail due to inherent dose-related toxicity (e.g. whole cell pertussis, cholera, typhoid vaccines) [10,11] and by WHO recommendations that manufacturers discuss the need for testing with the relevant NRA [4,5]. Likewise, the FDA previously added an administrative procedure allowing for complete exemption from the GST requirement [12]. While this addressed some vaccine-

* Corresponding author. Pfizer Vaccine Immunotherapeutics, Ottawa Laboratories, 340, Terry Fox Drive, Suite 200, Ottawa, ON K2K 3A2, Canada. Tel.: +1 613 254 5207; fax: +1 613 254 5625.
E-mail address: michael.mccluskie@pfizer.com (M.J. McCluskie).

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Table 1
Comparison of test conditions for the general safety, abnormal toxicity and innocuity tests.

Parameter	General safety test[a]	Abnormal toxicity test[b]	Innocuity test[c]
Animal weight	<22 g (2 mice) <400 g (2 guinea pigs)	17 to 24 g (5 mice) 250 to 400 g (2 guinea pigs)	17 to 22 g (5 mice) 250 to 350 (2 guinea pigs)
Mouse dosing	0.5 mL i.p.[d]	1 human dose i.p. <1.0 mL	1/2 human dose i.p. ≤1.0 mL
Guinea pig dosing	5.0 mL i.p.	1 human dose i.p. <5.0 mL	1 human dose i.p. ≤1.0 mL
Observation	7 days	7 days	7 days
Acceptance criteria	(1) Survive the test period (2) Do not exhibit any response which is not specific for or expected from the product and which may indicate a difference in its quality. (3) Do not weigh less at the end of the test period that at the time of injection.	No animal shows signs of ill health	No animal dies within 7 days or shows significant signs of toxicity

[a] US Food and Drug Administration. rev. 2013. U.S. Food and Drug Administration's Code of Federal Regulations (CFR), title 21, part 610: General biological products standards.
[b] European Pharmacopoeia (8.0). 2013. Chapter 2.6.9: Abnormal toxicity.
[c] World Health Organization. 1990. Expert Committee on Biological Standardization. Fortieth report. Technical report series. Vol. 800 (Annex. 2) Geneva, Switzerland: WHO.
[d] i.p. = intraperitoneal injection.

related issues, it did not address novel adjuvants that often directly activate the immune system, in which case required doses can result in strong systemic immune activation with resulting morbidity/mortality. One example is the toll-like receptor 9 (TLR9) agonist CpG being developed as vaccine adjuvant alone or combined with other adjuvants such as aluminum hydroxide (alum). CpG doses >5 mg/kg have caused severe clinical signs in non-human primates [13]. CpG doses in vaccines tested in humans have ranged from ~0.5–3 mg, administered in 0.5 or 1 mL [14–16]. If test guidelines were followed, this could result in mice and guinea pig doses of up to 3–15 mg (~150 and 60 mg/kg, respectively); much higher than typical CpG adjuvant doses (0.01–0.1 mg; 0.5 and 0.4 mg/kg) in these species [17,18]. Thus, the GST with adjuvant concentrations formulated for humans could give false positive results, compromising the intent of the test to assess for toxicity unrelated to the product. Herein, we describe studies evaluating the safety tests for the CpG/alum adjuvant combination, further demonstrating the limitations of the GST, ATT and IT and supporting alternative testing methods.

2. Materials and methods

2.1. Test articles

Alhydrogel “85” (Brenntag Biosector, Frederikssund, Denmark) was the source of alum. The B Class CpG ODN (CpG) (5′ TCG TCG TTT TTC GGT GCT TTT 3′) was synthesized with a nuclease-resistant phosphorothioate backbone (Avecia, Milford, MA) [19]. A single CpG/alum stock solution was prepared (4 mg/mL CpG, 2 mg/mL alum) to mimic adjuvant drug product concentration, which was diluted two-fold when combined with antigen. Stock solution was stored at 2–8 °C until required, then diluted with 12.5 mM Histidine, 187.5 mM NaCl, pH 6.5 to achieve final concentrations and stored at room temperature.

2.2. Animals

Hartley Guinea Pigs (<400 g) and CD-1, Swiss Webster, BALB/c mice (<22 g) (Charles River Laboratories, Montreal, QC, Canada); TLR9 knock-out (KO) (background C57/BL6) and C57/BL6 mice (both <22 g) (Taconic Farms, Germantown, NY, USA). All procedures performed on animals were in accordance with regulations and guidelines reviewed and approved by the Pfizer Institutional Animal Care and Use Committee and were conducted in facilities fully accredited by AAALAC International.

2.3. Dosing of animals

Guinea pigs and mice (3–13/group) received a single intraperitoneal bolus injection of CpG/alum; total volume of 5 (guinea pigs) or 0.5 mL (mice) as per FDA GST. Maximum adjuvant concentrations for animals aligned with those in our human vaccines: CpG at 2 mg/mL and alum at 1 mg/mL. Lower concentrations were tested when necessary to identify doses that could pass the test requirements. Animals were weighed prior to dosing and on day 7, and monitored daily for survival and clinical observations.

2.4. Statistical analysis

Data analysis used GraphPad Prism (GraphPad Software, San Diego, CA). Change in body weight was represented as mean change in % body weight (±SD) for each dose group.

3. Results and discussion

While similarities exist between the GST, ATT and IT, differences in dose levels, animal numbers and weights remain (Table 1). Furthermore, no guidance is given to animal strain; thereby allowing use of inbred or outbred animals. Our first attempt to conduct the GST with CpG/alum resulted in all guinea pigs being euthanized. Therefore, we investigated whether any of the safety tests could be used as prescribed supporting the release of the CpG/alum drug product for an experimental vaccine [20]. At dose volumes specified under GST guidelines and our maximum clinical adjuvant concentration (2 mg/mL CpG), mice and guinea pigs would receive 1 mg and 10 mg doses of CpG, respectively.

In guinea pigs, we performed a dose escalation starting at 1 mg due to our previous GST failure. Up to 4 mg CpG was tolerated and the adjuvant drug product would have passed the ATT and IT that only require the full human dose (i.e., 1 mg). However, at the 5 mg CpG dose, as required by the GST, guinea pigs demonstrated weight loss and ill health (Table 2), and would have failed the test.

In a parallel study, we tested common inbred mouse strains (C57BL/6, BALB/c). At 1 mg CpG; body weight was deceased in all mice over the test period and would be considered a failure by all three tests. This was considered CpG-mediated since TLR9 KO mice all gained weight and would have passed the test. In addition, CpG/