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Inflammation and immunogenicity limit the utility of the rabbit as a nonclinical species for ocular biologic therapeutics

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ABSTRACT

The nonclinical safety evaluation of therapeutic drug candidates is commonly conducted in two species (rodent and non-rodent) in keeping with international health authority guidance. Biologic drugs typically have restricted species cross-reactivity, necessitating the evaluation of safety in non-human primates and thus limiting the utility of lower order species. Safety studies of cross-reactive ocular biologic drug candidates have been conducted in rabbits as a second toxicology species, despite the fact that rabbits are not a rodent species. Such studies are often confounded by the development of anti-drug antibodies and severe ocular inflammation, the latter requiring studies to be terminated prematurely for animal welfare reasons. Notably, these confounding factors preclude the interpretation of safety. Nonclinical toxicology programs should be designed with consideration of ethical animal use and 3Rs principles (Replacement, Reduction and Refinement). The experience of several pharmaceutical sponsors, demonstrating that toxicology studies of ocular (intravitreal and topical ocular) biologic drug candidates in the rabbit are of limited interpretive value, calls into question the utility of such studies in this species and indicates that such studies should not be conducted.

1. Introduction

Intravitreal (ITV) administration of biologic drugs has radically changed the treatment paradigm for many retinal diseases, including age-related macular degeneration (AMD), diabetic macular edema (DME), diabetic retinopathy (DR), and retinal vein occlusion (RVO) (Zarbin and Szirth, 2007; Glanville et al., 2014; Das et al., 2015). To enable the clinical use of these drugs, nonclinical studies are conducted to evaluate the potential efficacy and pharmacokinetic characteristics, and to define the safety profile in support of clinical trials. However, the nonclinical safety assessment of these molecules is often complicated by the development of ocular inflammation. When such inflammation becomes severe during repeat-dose nonclinical safety studies, dosing may need to be suspended or terminated for animal welfare reasons. Even in cases where studies can be completed, ocular inflammation compromises the interpretation of the pharmacokinetics and safety profile of the therapeutic candidate. Although such repeat dose-related ocular inflammation has not translated to the clinic with the ITV biologics approved by the FDA to date (ranibizumab, aflibercept and ocriplasmin [Hahn et al., 2015; Meredith et al., 2015; Sigford et al., 2015; Khanani et al., 2016; Mansour et al., 2016]), the development of ocular inflammation in nonclinical studies remains a significant concern for ocular biologics since it may limit the ability of sponsors to adequately assess the nonclinical safety of these molecules.

While regulatory guidance exists for other classes of therapeutics, such as oncology, there is no specific guidance for the nonclinical development of ocular drugs. The common framework for nonclinical toxicology assessment of novel therapeutics is described in The International Council for Harmonisation of

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Technical Requirements for Pharmaceuticals for Human Use (ICH) M3(R2), which specifies the evaluation of toxicity in rodent and non-rodent species (ICH, 2009). ICH S6(R1) contains recommendations for the nonclinical safety evaluation of novel biologics, and while it states that toxicology studies may be conducted in two species, it specifies that they must be relevant species (ICH, 2011). A relevant species is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope (ICH, 2011). Safety evaluations of biologics are often limited to a single species, typically the non-human primate (NHP), based on limited cross-reactivity with lower-order species such as dog, rat, and mouse. When it is a pharmacologically relevant species, the rat is the most commonly used second species. Differences in physiology between species add a layer of complexity to the selection of an appropriate species for the evaluation of ocular therapeutics (Rowe-Rendleman et al., 2014). Rodents (mice and rats) are of limited use for intravitreally-administered biologics due to small vitreal volume and the proportionally larger size of the lens, and the potential for damage to the lens following repeated ITV administration in these species. Rabbits have a similar overall eye size to cynomolgus monkeys and have been used for efficacy studies of a number of novel ocular drug candidates (e.g., Gille et al., 2016); additionally, rabbits are commonly used for topical ocular drug safety assessment. A number of sponsors have been asked by the FDA to conduct ITV toxicology studies in the rabbit despite an established risk for severe immunogenicity reactions in this species. However, the use of two non-rodent species (rabbit and NHP) for safety evaluation is not in line with the regulatory guidances referenced above and is contrary to the spirit of the 3Rs (Replacement/Reduction/Refinement) of animal use in scientific research.

Rabbits are not often used as a general toxicology species outside of ocular drug development, and the availability of reagents for rabbit biomarkers and cytokines is poor compared to rodent and NHP. Additionally, the propensity of rabbits to mount robust humoral responses to foreign antigens presents another potential limitation of this species (Hall and O'Connor, 1970a; Hall and O'Connor, 1970b; Parks et al., 1961; Pribnow and Hall, 1970; Pribnow et al., 1971; Brinkman et al., 1981; Stills, 1994). Although the eye is in some respects an immune privileged site, this does not mean that an immune response cannot occur in the eye. Characteristics of the ocular humoral immune response include relative isolation of the ocular surface (mucosal immune system) and intraocular compartments from the systemic immune system, and suppression of cell-mediated responses within the ocular environment (Streilein, 2003; Benhar et al., 2012; Forrester and Xu, 2012). However, the introduction of biologics into the ocular compartment not only compromises the physical barrier (i.e., the blood-ocular barrier) but can break tolerance and trigger humoral responses under pro-inflammatory conditions. In addition to the basic principles of immunology that govern T and B cell responses to foreign and self-proteins, the potential adjuvant effect of extrinsic factors (product or host cell related impurities) that are particular to the therapeutic protein or its administration should also be considered for ocular drugs. Thus, ocular immunity is a spatial phenomenon dependent upon co-localization of immune cells with antigen presentation and co-stimulation forming the link between innate and adaptive immunity (Meek et al., 2003).

In the current report, the recent experience of several sponsors with ITV and topical ocular administration of biologics in rabbits is described. The aggregate results confirm the common observation of a high incidence and severity of intraocular inflammation that compromised the objectives of the toxicology studies, therefore limiting the utility of the rabbit for safety evaluations of these ocular biologic drug candidates. Furthermore, the rapid development of anti-drug antibodies (ADAs) was also observed. As the field of toxicology evolves, increasing scrutiny of animal use is prompting the re-evaluation of what constitutes a relevant species for safety evaluation (Chapman et al., 2013). While limited-duration pharmacokinetic studies of ocular biologics in rabbits may be suitable, the present results suggest that the rabbit is not an appropriate species for safety evaluation of intravitreally- and topically-administered ocular biologic drugs, and is of limited value when employed as a replacement of the rodent as a second toxicology species.

2. Methods

The appropriate Institutional Animal Care and Use Committees approved all procedures involving animals prior to the initiation of all studies. A high-level summary of the test articles and study designs across Sponsors is presented in Table 1.

2.1. Genentech methods

G1 is a humanized bispecific $F(ab')_2$ monoclonal antibody intended for the treatment of AMD. G1 was administered by ITV injection (50 μL, bilaterally) once every other week (Days 1, 15, 29, 43) to male and female New Zealand White rabbits (n = 5/sex/group; vehicle control, low, mid, and high dose levels). The assessment of toxicity was based on clinical observations, body weights, ophthalmic examinations (using a slit lamp biomicroscope and indirect ophthalmoscope), intraocular pressure measurements, fundus imaging (ocular photography (OP)), full-field electroretinography (ffERG) at similar intervals following each dose administration. Additionally, serum for clinical pathology assessment (clinical chemistry, coagulation, and hematology) was collected from all animals at least twice during the predose phase [at least 1 week apart] and on Days 2, and 15 (predose). Serum for ADA analysis was collected from all animals on Days 1 (predose), 7, 15, and 29 and analyzed using a validated bridging enzyme-linked immunosorbent assay (ELISA). At necropsy, an examination of the external features of the carcass; external body orifices; abdominal, thoracic, and cranial cavities; organs; and tissues was performed. A comprehensive set of tissues was collected and processed for hematoxylin and eosin (H&E) stain, and analyzed microscopically.

2.2. Roche methods

R1 is a humanized bispecific monoclonal antibody intended for the treatment of AMD. R1 was administered by ITV injection (50 μL, right eye only) to male and female Dutch-Belted rabbits (n = 2/sex/group; vehicle, low, mid, and high dose groups). All animals were dosed on Day 1, but due to the development of severe ocular inflammation, only 2 of 4 animals in the low dose group, and 0 of 4 animals in the mid and high dose groups received their scheduled second dose of R1 on Day 15. The assessment of toxicity was based on clinical observations, body weights, ophthalmic observations (using a slit-lamp biomicroscope and indirect ophthalmoscope), intraocular pressure measurement, and clinical and anatomic pathology. Serum was collected for ADA evaluations predose and on Days 8, 15, and 17; samples were analyzed using a sandwich ELISA method. On Day 15, all high-dose group animals were euthanized at an unscheduled interval due to observations of severe ocular inflammation. All surviving animals were euthanized on Day 17 (two days after administration of the second dose of R1 to 2 low-dose group animals). A comprehensive set of tissues was collected and processed for H&E stain, and analyzed microscopically.