ELSEVIER

feature

Current challenges and opportunities in nonclinical safety testing of biologics

Sven Kronenberg[1], sven.kronenberg@roche.com, Andreas Baumann[2], Lolke de Haan[3], Heather J. Hinton[1], Jonathan Moggs[4], Frank-Peter Theil[5], Ian Wakefield[6] and Thomas Singer[1]

Nonclinical safety testing of new biotherapeutic entities represents its own challenges and opportunities in drug development. Hot topics in this field have been discussed recently at the 2nd Annual BioSafe European General Membership Meeting. In this feature article, discussions on the challenges surrounding the use of PEGylated therapeutic proteins, selection of cynomolgus monkey as preclinical species, unexpected pharmacokinetics of biologics and the safety implications thereof are summarized. In addition, new developments in immunosafety testing of biologics, the use of transgenic mouse models and PK and safety implications of multispecific targeting approaches are discussed. Overall, the increasing complexity of new biologic modalities and formats warrants tailor-made nonclinical development strategies and experimental testing.

Introduction

Industry experts gathered on 3–4 December 2012 in Basel for the 2nd Annual BioSafe European General Membership Meeting, where they shared experiences and insights into the nonclinical safety assessment of new biotherapeutic entities. The 135 scientists – mostly from a pharmacokinetics (PK), toxicology or pathology background – represented Europe-based biopharmaceutical companies such as Roche, Novartis, Pfizer, UCB Pharma, Amgen, MedImmune, Bayer, GSK and many more. The meeting was organized by the world's largest biotechnology trade association: Biotechnology Industry Organization (BIO), and hosted by Roche, Basel. Contact details of presenters can be found in Box 1. Delegates emphasized the value of being able to connect and exchange information on nonclinical safety assessment strategies for the development of biotherapeutics. The meeting covered several nonclinical safety issues from explaining the potential accumulation of polyethylene glycol (PEG)ylated proteins in tissues to dose setting for first-time-in-human clinical trials.

PEGylated therapeutic proteins and cellular vacuolization: defining the relevant disposition and safety questions and potential solutions

Conjugation of PEG to therapeutic proteins (TP) is widely used to improve PK properties of proteins [1]. PEGylated entities have a long and extensive safe use in consumer products and medicines and available data indicate toxicity only at very high parenteral doses essentially restricted to the kidney, which is the main excretory route for PEG [2]. Rob Webster (Pfizer) gave an overview of the analytical challenges faced in determining the biological distribution and fate of PEG from biotherapeutics. PEG characteristics generally make radiolabeling and mass spectroscopic techniques unsuitable, although gel electrophoresis has been useful [3]. Ted Parton (UCB) described the application of NMR to study PEG tissue and plasma concentration profiles and urine elimination in humans and rats with PEGylated TPs. This demonstrated that PEGylation of a molecule can fundamentally alter its biodistribution. An excretion study (mass balance) performed in rats using NMR techniques showed 83% measured and 91% extrapolated PEG recovery after 12 weeks.

PEG-associated vacuolization in macrophages (foam cells), predominantly within tissues comprising the reticuloendothelial system, is well documented and is without apparent toxicologic significance [4]. Following high PEG TP exposure,

1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/j.drudis.2013.08.003

BOX 1

Experts involved in the symposium and email contacts

PEGylated therapeutic proteins and cellular vacuolization

Expert	Contact details
Rob Webster, PhD	Pfizer, Cambridge, USA rob.webster@pfizer.com
Ted Parton, PhD	UCB–Celltech, Slough, UK ted.parton@ucb.com
Annamaria Brändli-Baiocco, Dr med. Vet.	Roche, Basel, Switzerland annamaria.braendli-baiocco@roche.com
Ian Wakefield, PhD	UCB–Celltech, Slough, UK ian.wakefield@ucb.com

Cynomolgus monkey as a preclinical species

Expert	Contact details
Adam Hey, PhD	Novartis, Basel, Switzerland adam.hey@novartis.com
Max Warncke, PhD	Novartis, Basel, Switzerland max.warncke@novartis.com
Jonathan Moggs, PhD	Novartis, Basel, Switzerland jonathan.moggs@novartis.com

Unexpected fast clearing mAbs: mechanisms, risk assessment and development implications

Expert	Contact details
Wolfgang Richter, PhD	Roche, Basel, Switzerland wolfgang.richter@roche.com
Stewart Jones, PhD	Novarits, Basel, Switzerland stewart.jones@novartis.com
Frank-Peter Theil, PhD	UCB Pharma, Braine-l'Alleud, Belgium Peter.Theil@ucb.com

Multispecific targeting: PK and safety implications

Expert	Contact details
Andreas Baumann, PhD	Bayer Pharma, Berlin, Germany andreas.baumann@bayer.com
Benno Rattel, PhD	Amgen Research, Munich, Germany brattel@amgen.com
Niels Jørgen Ø. Skartved	Symphogen, Lyngby, Denmark nsk@symphogen.com

Transgenic mouse models for specific questions in drug disposition and toxicology

Expert	Contact details
Rajni Fagg, PhD	GlaxoSmithKline, Ware, UK rajni.s.fagg@gsk.com
Lolke de Haan, PhD	MedImmune, Cambridge, UK dehaanl@medimmune.com
Michael Otteneder, PhD	Roche, Basel, Switzerland michael.otteneder@roche.com
Balaji Agoram, PhD	MedImmune, Cambridge, UK agoramb@medimmune.com
Antonio Iglesisas, PhD	Roche, Basel, Switzerland antonio.iglesias@roche.com

Immunosafety

Expert	Contact details
Tobias Manigold, MD	University Hospital Basel, Switzerland tobias.manigold@usb.ch
Andrea Kiessling, PhD	Novartis, Basel, Switzerland andrea.kiessling@novartis.com
Christian Münz, PhD	University of Zürich, Switzerland christian.muenz@uzh.ch
Matthew Baker, PhD	Antitope, Cambridge, UK matthew.baker@antitope.co.uk

vacuolated macrophages are seen in other organs and/or tissues, including the liver, kidney, urinary bladder and brain choroid plexus (CP), and are considered to reflect normal physiologic processing of foreign material by scavenger phagocytic cells, producing no apparent effect on cell function or viability (Fig. 1). Other cell types can display vacuolization after high PEG TP exposure including hepatocytes, urinary bladder, epididymis, adrenal cortex, synoviocytes, ciliary bodies of the eyes and CP of the brain.

Anna Brändli-Baiocco (Roche) presented a pathology view of cytoplasmic vacuolizations, which can result from abnormal catabolism, transport or secretion and/or uptake of indigestible or slowly digestible materials. For PEG TPs, dose- and duration-dependent PEG accumulation and cytoplasmic vacuolization can be nonspecific or frequently target-associated as described for renal tubular epithelial cells [5] and neurons without cellular damage or functional impairment. Following administration of 40 kDa PEG TPs cellular vacuolizations appear to show only intact (40 kDa) PEG as demonstrated using immunohistochemistry and confocal microscopy. PEG vacuolization was only partially reversible and not reversible in neurons but without an apparent effect on neuronal function (*i.e.* for nerve conduction velocity and Fluro-Jade® stain to detect degenerating neurons, manuscript in preparation).

Ian Wakefield (UCB) described CP epithelial cell vacuolization in primates following high, sustained PEG TP exposure. The vacuolization appears as an inert finding, probably representing engulfed nonmetabolized material with no pathology indicative of any toxicologic significance, and can represent physiologic clearance by cells having endocytic capability. 'No effect' exposure levels, exceeding clinical exposure, suggest a potential threshold effect for PEG-induced CP vacuolization. Resolution could be partly dependent on cell turnover. Overall, the absence of pathology associated with PEG-related vacuolization in animals and extensive clinical use over many patient years provides evidence of safety to support the use of PEG for PEGylated TPs. The genesis of vacuolization and the potential impact of slow or no reversibility requires further understanding.

The cynomolgus monkey as a preclinical species

Jonathan Moggs (Novartis) started the session describing how the recent paradigm shift in the genetic characterization of the cynomolgus monkey *via* deep DNA sequencing technologies [6–9] provides a comprehensive picture of