ELSEVIER

How do we assure the quality of biological medicines?

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Biological medicines are a rapidly growing area of interest to many pharmaceutical companies, large and small. Under a broad definition they include not only modern high-tech products, such as monoclonal antibodies, enzymes and cytokines, but also older well-established products, such as vaccines and blood products. Despite a long history of standardisation and control of biological medicines, and an elaborate system of licensing and regulation, problems still occur because of their complexity. This review includes historical and regulatory background and three examples of problems seen with biotherapeutics: streptokinase, heparin and TGN1412.

Introduction and historical background

'Assuring the quality of biological medicines' is the mission statement of The National Institute for Biological Standards and Control (NIBSC), (see footnote [1]) which was formed along with the NBSB because of a result of an Act of Parliament in 1975, *The Biological Standards Act*. In describing aspects of the standardisation and control work of NIBSC and links to other organisations worldwide, we will aim to develop a useful overview of the work that goes into the licensing, standardisation and testing of biological medicines (see Box 1).

The history of biological standardisation and control of therapeutic products as practiced today may be traced back to the work of Paul Ehrlich in Germany in the 1890s as the director of the State Institute for Serum Research and Control in Berlin. The system he devised for routine batch testing by comparison with a standard of known potency is outlined in his publication, 'The Potency Estimation of Diphtheria Antiserum and its Theoretical Basis'. In the early 1900s, the range of antisera and vaccines increased and legislation was established in Europe, for example in the UK, The Therapeutic Substances Act in 1925 and, in the US, the 1902 Biologics Control Act, to regulate the production, licensing, sale and importation of vaccines, sera and related products. In the UK, the role of overseeing the quality of biologicals was originally performed by the MRC (see footnote 1) until it was handed over to the NBSB in 1975. The FDA (see footnote 1) celebrated its centenary in 2006 and several helpful resources describing the history of the organisation are available (http://www.fda.gov/cdrh/fdaandyou/issue09.html). Nowadays biological medicines are dealt with by one of two sections within the FDA, CBER (see footnote 1) or CDER (see footnote 1). CBER handles more complex medicines, including blood products, vaccines, cells and gene therapy products. CDER is responsible for biopharmaceuticals that, though they may be biotechnology products, are easier to characterise than many blood products or vaccines, for example.

It is recognised that the potency of complex biologicals needs to be tested by comparative methods against a stable standard. In this way it is not necessary to define test methods rigorously and express absolute responses. Rather, it is the ratio of the activity of the test batch *versus* the standard that can be used to determine the potency of the test preparation. This approach contrasts with alternative, earlier, highly unreliable systems, for example, the potency of digitalis was sometimes expressed in 'frog units' as the amount required to kill 1 g of frog [1]. The MRC also has a long history of preparing and distributing stable freeze-dried biological standards that goes back to the 1st International Standard for Insulin, 1925, prepared by Henry Dale [2]. The task of preparing and distributing International Standards (IS) worldwide is a major responsibility of NIBSC, which holds >95% of all IS on behalf of WHO (see footnote 1). Biological standardisation was recognised as a priority by the Health Committee of the League of Nations in

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[1] Acronyms are common in the area of regulation; a listing of those associated with the work described in this review is presented in Box 1.

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BOX 1

List of abbreviations and acronyms, used in this article and in the field of regulation of biologicals

BRP	Biological Reference Preparation (from EDQM)
BP	British Pharmacopoeia
BSHT	British Society for Haemostasis and Thrombosis
CBER	Center for Biologics Evaluation and Research (of FDA)
CDER	Center for Drug Evaluation and Research (of FDA)
CHMP	Committee for Medicinal Products for Human Use (of EMEA)
EDQM	European Directorate for the Quality of Medicines
EMEA	European Medicines Evaluation Agency
ECBS	Expert Committee on Biological Standardisation (of WHO)
EP	European Pharmacopoeia
FDA	Food and Drug Administration
IS	International Standard
IU	International Unit
MHRA	Medicines and Healthcare Products Regulatory Agency
MRC	Medical Research Council
NBSB	National Biological Standards Board
NIBSC	National Institute for Biological Standards and Control
OMCL	Official Medicines Control Laboratory
UN	United Nations
WHO	World Health Organisation

1921, and later this responsibility passed on to the UN (see footnote 1) and WHO. The ECBS (see footnote 1) of the WHO began to establish standards in 1947 by the process that is familiar today. Technical Reports from the ECBS describing the establishment, discontinuation and replacement of WHO IS, as well as guidelines and recommendations, are available from WHO (http://www.who.int/biologicals/expert_committee/en/).

WHO IS are prepared at NIBSC in sealed glass ampoules and define the potency of a biological material in International Units (IU). IS receive the final approval of the Director General of WHO following recommendation from the ECBS, and member nations of the UN agree to implement these units. Before this, IS will have taken several years to make and calibrate, work which involves an international collaborative study including many expert laboratories around the world. Data will have been carefully scrutinised by biostatisticians, and numerous expert scientists and learned societies. NIBSC currently has a catalogue of around 600 IS and other reference materials amounting to around 2 million ampoules held in freezers at −20 °C for long-term storage (http://www.nibsc.ac.uk/products/). These are shipped worldwide to scientists in academia, regulatory authorities and pharmaceutical companies at a rate of around 100,000 ampoules a year.

Definitions

As discussed above, comparative tests are used to quantitate biological potencies when physicochemical methods are not appropriate. This may be a temporary situation, however, and some biologicals may become amenable to full characterisation by physical and chemical methods as technology advances, as has been the case with antibiotics and some hormones. So, the definition of a biological can change over time. Another complicating issue surrounding biologicals is the range of different terms and their inconsistent use. For example, in some cases terms like biotherapeutic, biotechnology product, biological medicine and biopharmaceutical may be used interchangeably, but in other circumstances they may have subtly different meanings. Definitions used by regulatory authorities such as the FDA and EMEA (see footnote 1) can be found on their websites (http://www.fda.gov/oia/biolog.html and http://www.emea.europa.eu/index/indexh1.html). Table 1 gives a breakdown of product types. For the purposes of quality control and safety, regulatory organisations such as NIBSC are certainly interested in the broad definition of biopharmaceuticals which includes low-tech and high-tech products.

European regulatory framework

A mature system of licensing, batch release and post-marketing surveillance is established in Europe [3]. This is represented schematically in Figure 1, which also highlights the interactions of NIBSC with the MHRA (see footnote 1) within the UK and with other European organisations, including the EMEA and EDQM (see footnote 1). NIBSC provides expert input on working parties within the EMEA for licensing purposes (e.g. the Biologics, Blood Products and Gene Therapy Working Parties, which feed into the CHMP (see footnote 1)) and at the EDQM to Expert Groups, 6 for Biological Substances, 6B for Blood Products and 15 for Sera and Vaccines. These Expert Groups at the EDQM advise on drafting guidelines and monographs for the European Pharmacopoeia, which has legal status within Europe and is extensively used worldwide. Batch release of blood products and vaccines is also a highly coordinated effort in Europe, organised by the EDQM through a network of OMCLs (see footnote 1), which has representation from all EU member states and associated countries, such as Switzerland, Norway and Iceland. NIBSC is the UK representative in the OMCL network for the control of biologicals.

Despite our extensive systems of regulation, licensing, standardisation and control, problems will arise. In the following sections of this review, three examples of problem areas are discussed.

TABLE 1

Pharmaceutical product classifications.

Pharmaceutical			
Drug	**Borderline**	**Biopharmaceuticals**	
		Low-tech	**High-tech**
Small chemical entities	Peptides	Vaccines	Monoclonal antibodies
	Polynucleosides	Blood products	Recombinant proteins
	Fermentation products	Toxins	Gene therapy
	Semisynthetic products	Antisera	Stem cells

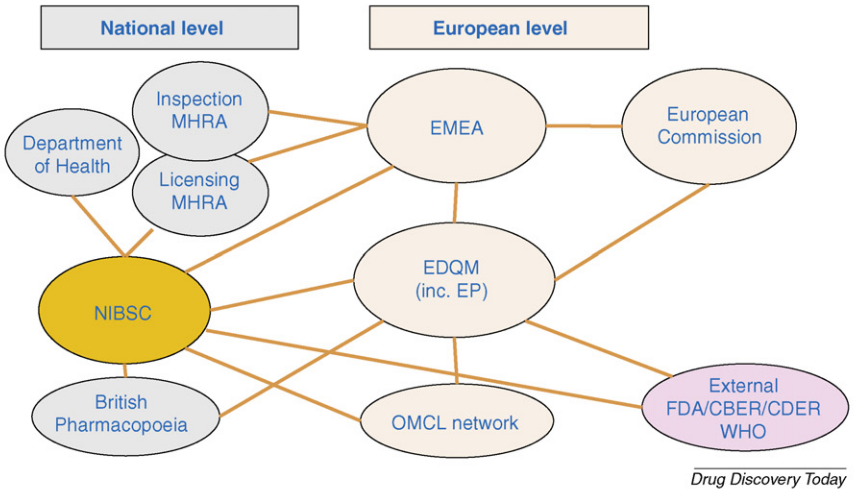

FIGURE 1

Regulatory framework in the UK and Europe highlighting NIBSC involvement. A list of abbreviations is also provided in Box 1. NIBSC provides a major source of expertise in the licensing of biological drugs, either at national level in conjunction with the MHRA, or at European level by providing expert input to the EMEA through several specialist working parties. NIBSC scientists may also represent the UK on expert groups drafting monographs for the EP at the EDQM. NIBSC is also part of a European network of laboratories (OMCL) that monitor and batch release blood products and vaccines to ensure that each batch conforms to guidelines and adheres to licence specifications. The work of NIBSC as the manufacturer and custodian of most of the worlds IS is also highlighted through links with WHO and the EDQM and the FDA, which regularly collaborate in the generation of these reference materials.

Biosimilars

Biosimilars, biogeneric, generic biopharmaceutical, biocomparable and follow-on biopharmaceutical are all terms associated with the contentious area of approving generic biologicals as alternatives to the first licensed product (for more discussion, see [4]). In the case of simple chemical entities, it is reasonable to follow an abbreviated licensing path if the alternative chemical active ingredient is clearly the same as the original. When drugs become increasingly complex, as is obviously the case with biologicals, the comparison becomes technically more difficult. The approach taken with biosimilars is, however, notably different in Europe and in the US, with the EMEA taking a more liberal line [5,6]. A guideline on *Similar Biological Medicinal Products* from the CHMP has been published, summarising the approach adopted by the EMEA [7]. The complexity of the product is crucial and it is much easier to consider highly purified biotechnology-derived products than older products extracted from biological sources. Expert opinion currently holds that immunologicals such as vaccines are unlikely to be considered for licensing as biosimilars with an abbreviated dossier; and for blood or plasma-derived products it would *not* be possible. Even recombinant alternatives to plasma-derived products fall into this category because of their complex and variable physicochemical, biological and functional characteristics. In the manufacture of biologicals it is said that 'the process is the product' and a clear illustration of this view is provided in the case of streptokinase.

Streptokinase

Streptokinase is a small plasminogen-binding protein produced by *Streptococcus haemolyticus*, used successfully for many years as a thrombolytic or 'clot buster' [8,9] to treat myocardial infarction. The established dose is 1.5 million IU infused over a period of 90 min and the unit is defined currently by the 3rd IS for Streptokinase, established in 2002 [10]. The traditional expression system for streptokinase is from streptococcus; however, several biotech companies have begun production in *E. coli*, especially in developing countries where we have already identified quality issues [11,12]. The extent of the problems we found with recombinant streptokinase was, however, surprising. Figure 2 shows results from potency determinations of four batches of recombinant streptokinase measured against the 3rd IS for streptokinase in three different assay systems and indicate that the presence of fibrin could change the potency up to three- to fourfold. This was unexpected, based on experience from our collaborative study [10] where it was found that the presence of fibrin made no difference and allowed the simplification of the EP (see footnote 1) method to remove the requirement for a fibrin matrix [13]. Currently, the full explanation for these large assay discrepancies is not known, but one hypothesis focuses on the N-terminal amino acid residue in streptokinase, which is known to be important for plasminogen binding and activation [14] by a mechanism termed 'molecular sexuality' [15]. The N-terminal Ile and the first 60 amino acids have been targeted in several studies [16,17]. Thus, problems may arise where processing of the N-terminal sequence is not 100% efficient, and there may be a significant amount of N-terminal Met with a different profile of activity from native Ile-1 streptokinase. There is a danger that the same streptokinase product may have three to four times more activity if measured in the presence of fibrin, as in the Indian Pharmacopoeia rather than in the absence of fibrin as in the European Pharmacopoeia, following revision of the method in 2005 [18] where detailed analysis of results from the collaborative study to establish the 3rd IS for Streptokinase [10] showed that fibrin was not necessary where test and IS Streptokinase were identical. In circumstances where

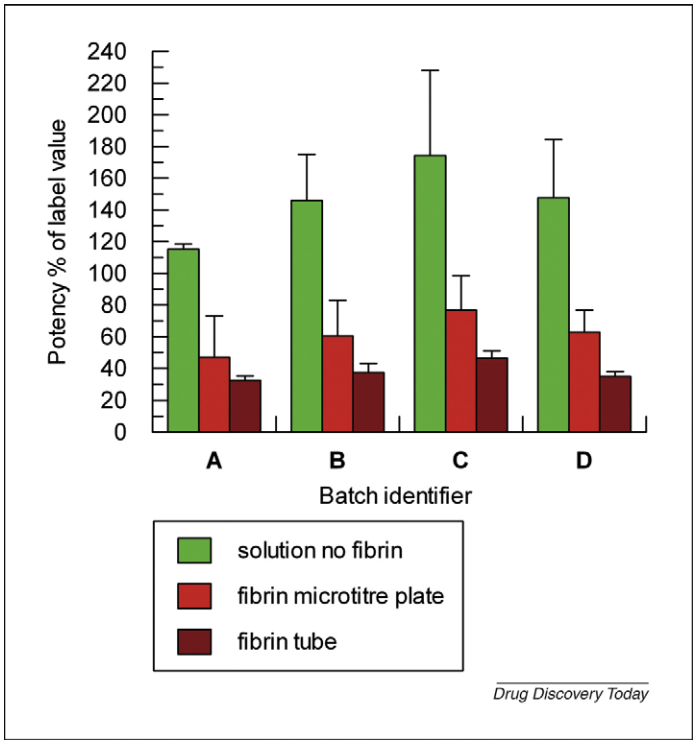

FIGURE 2

Potency estimates for four batches of recombinant streptokinase (A–D) versus the 3rd IS for Streptokinase (00/464, NIBSC) in three different assay formats. The current EP (and BP) method does not include fibrin and is a chromogenic plasminogen activation assay in solution. Earlier pharmacopoeial methods included a fibrin matrix and two assay variations are shown that include a fibrin substrate using a microtitre plate format or in tubes. Native streptokinase gives consistent results (not shown) but recombinant streptokinase potencies are highly dependent on the assay format.

recombinant streptokinase is not identical to IS Streptokinase, because of incomplete processing, it may not be possible to assign potency values to recombinant streptokinase products because there appears to be no appropriate IS and they cannot be considered simple biosimilars.

Heparin

Unfractionated heparin (UFH) was first isolated from liver by McLean in 1916 and the name 'heparin' was coined by Howell and Holt in 1918. As a complex heterogeneous polysaccharide, heparin requires a combination of physicochemical and biological tests to assure adequate quality control. The first clinical trial in humans was carried out by Murray in 1935 [19] and UFH has been used successful since then as an anticoagulant. The first IS for anticoagulant potency of UFH was established by the League of Nations in 1941 and replacement of the current 5th IS has recently been initiated. The major side-effect of heparin use is thrombocytopaenia, a condition typified by either transient depletion of platelets or production of autoimmune antibodies against platelet factor 4 and this occurs in 1–3% of heparinised patients [20]. Allergic reactions to heparin are rare [21–23], but in recent months (November 2007–March 2008) there have been reports of severe acute allergic reactions with hypotensive complications clustered in the US and Germany. To date, 131 deaths have been reported in the US. Over-sulphated chondroitin sulphate, a semisynthetic contaminant, has been identified as the cause of these adverse reactions [24]. *In vitro* and *in vivo* studies suggested that the allergic and hypotensive symptoms could be caused by the activation of prekallikrein leading to the liberation of bradykinin from high molecular weight kinnogen [25]. Investigations indicated that apart from the failure to ensure good manufacturing practice (GMP) and traceability of the heparin supply chains, the current pharmacopoeial tests and specifications may be inadequate to ensure the purity and safety of heparin and heparin-related products. As an immediate response to the crisis, the FDA introduced two mandatory tests, ^{1}H NMR and capillary electrophoresis for screening of heparin active pharmaceutical ingredient (API) for over-sulphated chondroitin sulphate [26,27]. These two tests, however, may not be able to detect other possible contaminants, such as chitosan sulphate and fucoidan. Considering that UFH APIs are also used as starting materials for other therapeutics, such as low molecular weight heparins, heparin-coated medical devices and heparin for non-anticoagulant indications, there is an urgent need to revise, upgrade and harmonise pharmacopoeial monographs for heparin therapeutics to prevent and minimise further heparin-related adverse events. The relatively easy-to-implement steps are the revision of specifications of validated tests already in the current EP monograph for heparin. For example, the optical rotation specification is +35°, if it is raised above +50°, it will minimise the presence of negatively rotated contaminants and impurities such as over-sulphated chondroitin sulphate and dermatan sulphate. Increasing the limit of specific activity from not less than 150 IU/mg to 200 IU/mg should also be considered. Replacement of plasma-based potency assays with antithrombin-dependent methods, such as the antithrombin-dependent anti-factor IIa assay, would also make the spiking of heparin cofactor II dependent contaminants less attractive. In addition, if an anti-factor Xa activity assay is introduced, the ratio of anti-Xa and anti-IIa activity can also be a useful quality assurance parameter for unfractionated heparin. Other tests for quantification of impurities such as chondroitin sulphate and dermatan sulphate would also help to ensure the purity of heparin, but there is no indication what other contaminants may be found in heparin products in the future. Therefore, it is not possible to design specific tests for contaminants, but several measures can be proposed to assure the quality and purity of heparin.

TGN1412

In March 2006 news of a disastrous phase I clinical trial hit the headlines when six volunteers developed life-threatening complications, including fever, pain, hypotension, disseminated intravascular coagulation and organ failure, after receiving infusions of a CD28-specific monoclonal antibody, TGN1412 [28,29]. This novel therapeutic was a humanised IgG4 monoclonal antibody that was engineered to be a superagonist to the CD28 receptor on T lymphocytes. Normal T cell activation requires two signals, one from a specific foreign antigen and a second ligand that binds to CD28 as a general 'go' signal, but TGN1412 was designed to override the requirement for both signals and to activate all T cells with the CD28 receptor. In animal models, treatment with TGN1412 orthologues leads to selective activation of regulatory T cells and suppression of inflammatory autoimmune disease [30]. It was expected that TGN1412 would be an effective treatment for B

cell chronic lymphocytic leukaemia and rheumatoid arthritis in humans. The antibody had been problem-free in preclinical safety tests, including *in vitro* testing involving human white blood cells and in preclinical animal studies, so the clinical trial was duly licensed by the MHRA and carried out at Northwick Park Hospital by Parexel. Adverse events were apparent in all TGN1412 recipients within 90 min of administration but absent in the two subjects receiving placebo. The trial was halted and remaining clinical materials transferred to NIBSC for testing along with other production batches and reference materials from the manufacturer, TeGenero. Initial investigations were able to show that the product used in the trials was free from contamination with endotoxin, was of the quality expected and comparable to the batch used in animal testing. Thus, the mechanism underlying the extreme adverse reactions was not clear and further investigations were carried out. It became apparent from *in vitro* experiments with human lymphocytes that antibody presentation was a crucial factor [31]. Only when TGN1412 was immobilised by drying onto a surface, or coupled *via* immobilised anti-Fc antibody, or co-cultured in the presence of an endothelial cell monolayer were responses seen in cultured lymphocytes. These included cell proliferation and release of cytokines, TNF-α, IL-2, IL-6, IL-8 and IFN-γ. No stimulation was observed when TGN1412 was incubated with cells in solution or when cross-linked by anti-Fc antibodies in solution. These findings explain to some extent why the initial *in vitro* safety testing carried out during the development of TGN1412 missed the potential dangers of the antibody *in vivo*. Administration of TGN1412 to trial volunteers seems to have induced the rapid release of a cascade of pro-inflammatory cytokines (cytokine storm), which then led to a longer term phase of cardiovascular shock, acute respiratory distress and multiple organ failure. *In vitro* studies at NIBSC also demonstrated that white blood cells from macaques, the animals used in preclinical studies, did not respond in the same way to immobilised TGN1412 as did human cells. This may not have been expected because the extracellular domain of Cynomolgus macaque CD28 is 100% identical to the human receptor (agreed after some debate, see [32,33]) and there does appear to be binding and some response to appropriately immobilised TGN1412 by cultured macaque cells. This response is, however, limited and the difference between human and macaque cell responses may be at the signalling level, or by some other unknown mechanism [31]. Another feature that emerged from *in vitro* studies with cultured human lymphocytes was the bell-shaped proliferative dose response over a concentration range of immobilised TGN1412. Calculations from these studies suggested that the human volunteers might have in fact received close to the optimum dose for immunostimulation during the phase I trial. The starting dose of the phase I clinical trial of TGN1412 was based on a 'No observed adverse effect level' (NOAEL) of 50 mg/kg defined in a Cynomolgus macaque toxicology study and draft FDA guidelines 'Estimating the Safe Starting Dose in Clinical Trials for Therapeutics in Adult Healthy Volunteers'. Using a default safety factor of 10 a maximum recommended starting dose of 1.6 mg/(kg day) was calculated, but a more conservative starting dose of 0.1 mg/(kg kg) was proposed and finally used. These calculations were, however, flawed because none of these doses produce any adverse effects in Cynomolgus macaques [28].

Following the trial there was a great deal of analysis of what went wrong and what needs to be done to prevent future disasters. Proposals for dealing with the risk of novel drugs with unknown mechanisms have been discussed at length [34] and the extra dangers associated with agonist drugs targeting the immune system have also been noted [35,36]. The events surrounding this phase I trial and subsequent recommendations were presented in the report from the Expert Scientific Group on Phase One Clinical Trials [37] (see also [38]), and Guidelines have also been developed by the EMEA to formalise the conduct of clinical trials of potentially high risk medicinal products [39]. These types of recommendations will improve safety and also inevitably have costs in terms of time and money and will result in the slowing of the development of novel biotherapeutics, at least in the short term.

Conclusions

As the developer and custodian of most of the WHO IS for biological medicines, NIBSC has a unique role in not only working with regulatory organisations but also providing help and advice to the pharmaceutical industry. The development of IS, batch release work as a European control laboratory and interactions with industry allow scientists from NIBSC to establish wide ranging expertise that may be called upon in a variety of situations that challenge public health. Three such examples have been discussed to highlight such problems and show how cutting edge scientific research can be used to tackle new problems in old established biological drugs or gain insights into unforeseen problems in novel therapeutics. Biological medicines new and old will continue to throw up new questions and we must respond with flexibility and high calibre science to assure quality and safety.

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