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Review article

Pharmaceutical biotechnology products approved within the European Union

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Abstract

The manufacture of therapeutic proteins represented the first true industrial application of recombinant DNA technology. Thus far some 88 recombinant proteins/monoclonal antibody-based products have gained marketing approval within the European Union (EU). This represents 36% of all new drug approvals since the introduction of the new centralized European drug approval system in 1995. More recently, an increasing proportion of approved proteins are engineered, tailored to display altered pharmacokinetic profiles or reduced immunogenicity in man. Currently no nucleic acid-based products are approved in the EU. Technical innovations/milestones likely characterizing the biopharmaceutical industry within the next decade include approval of some products produced in transgenic systems, approval of some products administered by non-parenteral means, approval of at least some nucleic acid-based products and the identification of novel biopharmaceuticals/biopharmaceutical targets through discoveries in functional genomics and proteomics.

Keywords: Biopharmaceutical; Therapeutic protein; Pharmaceutical biotechnology; European Medicines Evaluation Agency

1. Introduction

The development of recombinant DNA technology in the mid 1970s marked the beginning of the modern biotechnology era. The manufacture of therapeutic proteins represented the first true industrial application of this technology [1]. The first such recombinant therapeutic protein (insulin) was approved for general medical use only 21 years ago. Today there are in excess of 100 such products approved in some world region at least, with 88 having received approval within the European Union (EU).

During the 1980s the term 'biopharmaceutical' became synonymous with 'therapeutic protein produces by recombinant DNA technology' (or, in the case of a small number of therapeutic monoclonal antibodies, 'by hybridoma technology'). Clinical evaluation of nucleic acid-based drugs used for the purposes of gene therapy and antisense technology commenced in the 1990s, and today the term biopharmaceutical also encompasses such (as yet experimental) drugs [2].

This mini-review aims to provide a summary overview of the biopharmaceutical products thus far approved for general medical use within the EU. In the context of its publication organ (the European Journal of Pharmaceutics and Biopharmaceutics) it is included in this, the inaugural issue of the 'pharmaceutical biotechnology' section of the journal. In this context the article aims to provide a benchmark for regular readers, allowing them to place future articles detailing innovations in the pharmaceutical biotechnology arena in the context of the portfolio of biopharmaceuticals already approved for use. Reflecting the broad range of expertise of the readership, the article had been written at a level appropriate for the interested reader, even if their expertise does not pertain to pharmaceutical biotechnology.

2. European Union approval procedures

Since the mid 1980s a substantial body of harmonizing pharmaceutical legislation has been adopted throughout the EU. Published as the nine volume series 'the rules governing medicinal products in the European Union' [3], enactment of this legislation has facilitated the introduction of a common EU system for the authorization and subsequent supervision of medicinal products. The European Medicines Evaluation Agency (EMEA, Canary Warf, London) was created to coordinate and manage this drug approval system, and it began its work in February 1995 [4].

Central to the working of the EMEA are two technical

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Table 1
Biopharmaceuticals (recombinant proteins, monoclonal antibody or nucleic acid-based products) thus far approved in the European Union via the centralized procedure[a]

Product	Marketing authorization holder	Therapeutic indication (year first approved)
Recombinant blood factors/blood related products		
Benefix (rhFactor IX produced in CHO cells)	Genetics Institute	Haemophilia B (1997)
Kogenate (rhFactor VIII produced in BHK cells. Also sold as Helixate by Centeon via a license agreement)	Bayer	Haemophilia A (2000)
Helixate NexGen (octocog alfa; rhFactor VIII produced in BHK cells	Bayer	Haemophilia A (2000)
NovoSeven (rhFactor VIIa produced in BHK cells)	Novo-Nordisk	Some forms of haemophilia (1995)
ReFacto (moroctocog-alfa, i.e. B-domain deleted rhFactor VIII produced in CHO cells)	Genetics Institute	Haemophilia A (1999)
Ecokinase (reteplase, rtPA; differs from human tPA in that 3 of its 5 domains have been deleted. Produced in *E. coli*)	Galenus Mannheim	Acute myocardial infarction (1996)
Rapilysin (reteplase, rtPA; see Ecokinase)	Boehringer Manheim	Acute myocardial infarction (1996)
Tenecteplase (also marketed as Metalyse) (TNK-tPA, modified rtPA produced in CHO cells)	Boehringer Ingelheim	Myocardial infarction (2001)
Refludan (anticoagulant; recombinant hirudin produced in *S. cerevisiae*)	Behringwerke AG	Anticoagulation therapy for heparin-associated thrombocytopenia (1997)
Xigris (drotrecogin-α; rh activated protein C produced in a mammalian (human) cell line	Eli Lilly	Severe Sepsis (2002)
Recombinant hormones		
Humalog (insulin lispro, an insulin analogue produced in *E. coli*)	Eli Lilly	Diabetes Mellitus (1996)
Insuman (rh-Insulin produced in *E. coli*)	Hoechst AG	Diabetes Mellitus (1997)
Liprolog (Bio Lysprol, a short-acting insulin analogue produced in *E. coli*)	Eli Lilly	Diabetes Mellitus (1997)
NovoRapid (insulin Aspart, short acting rhInsulin analogue	Novo Nordisk	Diabetes Mellitus (1999)
Novomix 30 (contains insulin Aspart, short acting rhInsulin analogue (see NovoRapid) as one ingredient	Novo Nordisk	Diabetes Mellitus (2000)
Actrapid/Velosulin/Monotard/Insulatard/Protaphane/Mixtard/ Actraphane/Ultratard (all contain rhInsulin produced in *S. cerevisiae* formulated as short/intermediate/long acting product)	Novo Nordisk	Diabetes Mellitus (2002)
Lantus (insulin glargine, long acting rhInsulin analogue produced in *E. coli*)	Aventis pharmaceuticals	Diabetes Mellitus (2000)
Optisulin (insulin glargine, long acting rhInsulin analogue produced in *E. coli*, see Lantus)	Aventis pharma	Diabetes Mellitus (2000)
Glucagen (rhGlucagon produced in *S. cerevisiae*)	Novo Nordisk	Hypoglycemia
Thyrogen (thyrotrophin-α, rhTSH produced in CHO cells)	Genzyme	Detection/treatment of thyroid cancer (2000)
Nutropin AQ (rhGH produced in *E. coli*)	Schwartz Pharma AG	Growth failure, Turners syndrome (2001)
Gonal F (rhFSH produced in CHO cells)	Serono	Anovulation and Superovulation (1995)
Puregon (rhFSH produced in CHO cells)	N.V. Organon	Anovulation and Superovulation (1996)
Luveris (lutropin alfa; rhLH produced in CHO cells)	Ares-Serono	Some forms of infertility (2000)
Ovitrelle also termed Ovidrelle (rhCG produced in CHO cells)	Serono	Used in selected assisted reproductive techniques (2001)
Forcaltonin (r Salmon calcitonin produced in *E. coli*)	Unigene	Paget's disease (1999)
Cytokines		
Neorecormon (rhEPO produced in CHO cells)	Boehringer-Mannheim	Treatment of anaemia (1997)
Aranesp (darbepoetin alfa; long acting rEPO analogue produced in CHO cells)	Amgen	Treatment of anaemia (2001)
Nespo (darbepoetin alfa; see also Aranesp; long acting rEPO analogue produced in CHO cells)	Dompe Biotec	Treatment of anaemia (2001)
Neulasta (pegfilgrastim, r pegylated filgrastim. Also marketed as Neupopeg	Amgen	Neutropenia (2002)
Intron A (rIFN-α-2b produced in *E. coli*)	Schering Plough	Cancer, genital warts, Hepatitis (2000)
PegIntron A (PEGylated rIFN-α-2b produced in *E. coli*)	Schering Plough	Chronic Hepatitis C (2000)
Viraferon (rIFN-α-2b produced in *E. coli*)	Schering Plough	Chronic Hepatitis B & C (2000)
ViraferonPeg (PEGylated rIFN-α-2b produced in *E. coli*)	Schering Plough	Chronic Hepatitis C (2000)
Betaferon (rIFN-β-1b, differs from human protein in that Cys 17 is replaced by Ser. Produced in *E. coli*)	Schering AG	Multiple sclerosis (1995)
Avonex (rhIFN-β-1a, produced in CHO cells)	Biogen	Relapsing Multiple Sclerosis (1997)
Infergen (rIFN-α, synthetic type I interferon produced in *E. coli*	Yamanouchi Europe	Chronic Hepatitis C (1999)

Table 1 (*continued*)

Product	Marketing authorization holder	Therapeutic indication (year first approved)
Rebif (rh IFN-β-1a, produced in CHO cells)	Ares Serono	Relapsing/remitting Multiple Sclerosis (1998)
Alfatronol (rhIFN-α-2b produced in *E. coli*)	Schering Plough	Hepatitis B, C, and various cancers (2000)
Virtron (rhIFN-α-2b produced in *E. coli*)	Schering Plough	Hepatitis B & C (2000)
Vibragen Omega (rFeline IFN-ω)	Virbac	Veterinary; reduction in mortality/ symptoms of canine parvovirus (2001)
Beromun (rhTNF-α, produced in *E. coli*)	Boehringer-Ingelheim	Adjunct to surgery for subsequent tumor removal, to prevent or delay amputation (1999)
Revasc (anticoagulant; recombinant hirudin produced in *S. cerevisiae*)	Ciba Novartis Europharm	Prevention of venous thrombosis (1997)
Enbrel (rTNFR-IgG fragment fusion protein produced in CHO cells)	Wyeth Europa	Rheumatoid arthritis (2000)
Regranex (rhPDGF produced in *S. cerevisiae*)	Janssen-Cilag	Lower extremity diabetic neuropathic ulcers (1999)
Vaccines		
Tritanrix-HB (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Smithkline Beecham	Vaccination against Hepatitis B, diphtheria, tetanus and pertussis (1996)
Infanrix-Hep B (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Smithkline Beecham	Immunization against diphtheria, tetanus, pertussis and hepatitis B (1997)
Infanrix-Hexa (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Smithkline Beecham	Immunization against diphtheria, tetanus, pertussis, polio, *Haemophilus influenzae* b and hepatitis B (2000)
Infanrix-Penta (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Smithkline Beecham	Immunization against diphtheria, tetanus, pertussis, polio, and hepatitis B (2000)
Ambirix (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Glaxo SmithKline	Immunization against hepatitis A and B. (2002)
Twinrix, adult and pediatric forms in EU (combination vaccine containing rHBsAg produced in *S. cerevisiae* as one component)	Smithkline Beecham	Immunization against hepatitis A and B. (1996)
Primavax (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Pasteur Merieux MSD	Immunization against diphtheria, tetanus and hepatitis B (1998)
Procomvax (combination vaccine, containing rHBsAg as one component)	Pasteur Merieux MSD	Immunization against Haemophilus influenzae type B and hepatitis B (1999)
Hexavac (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Aventis Pasteur	Immunization against diphtheria, tetanus, pertussis, Hepatitis B, polio & *Haemophilus influenzae* type b (2000)
Triacelluvax (combination vaccine containing r(modified) pertussis toxin	Chiron SpA	Immunization against diphtheria, tetanus and pertussis (1999)
Hepacare (r S, pre-S and pre-S2 hepatitis B surface antigens, produced in a mammalian (murine) cell line	Medeva Pharma	Immunization against Hepatitis B (2000)
HBVAXPRO (rHBsAg produced in *S. cerevisiae*)	Aventis Pharma	Immunization of children & adolescents against hepatitis B (2001)
Porcilis porcoli (combination vaccine containing r *E. coli* adhesions)	Intervet	Veterinary; active vaccination of sows (1996)
Fevaxyl Pentofel (combination vaccine containing r Feline leukaemia viral antigen as one component)	Fort Dodge Laboratories	Veterinary; immunization of cats against feline pathogens (1997)
Porcilis AR-T DF (combination vaccine containing a modified toxin from *Pasteurella multocida* expressed in *E. coli*)	Intervet	Veterinary; active immunization of sows (2000)
Porcilis pesti (vaccine containing r classical swine fever antigen produced in an insect cell line)	Intervet	Veterinary; immunization of pigs against classical swine fever (2000)
Bayovac CSF E2 (vaccine containing r classical swine fever virus antigen produced in an insect cell line)	Bayer	Veterinary; immunization of pigs against classical swine fever (2001)
Monoclonal antibody-based products		
CEA-scan (Arcitumomab, murine Mab fragment (Fab), directed against human carcinoembryonic antigen, CEA)	Immunomedics	Detection of recurrent/metastatic colorectal cancer (1996)
Zenapax (Daclizumab, humanized Mab directed against the α chain of the IL-2 receptor)	Hoffman La Roche	Prevention of acute kidney transplant rejection (1999)
Simulect (Basiliximab, chimaeric Mab directed against the α chain of the IL-2 receptor)	Novartis	Prophylaxis of acute organ rejection in allogeneic renal transplantation (1998)
Remicade (Infliximab, chimaeric Mab directed against TNF α)	Centocor	Treatment of Crohn's disease (1999)

Table 1 (*continued*)

Product	Marketing authorization holder	Therapeutic indication (year first approved)
Synagis (Palivizumab, humanized Mab directed against an epitope on the surface of respiratory syncytial virus)	Abbott	Prophylaxis of lower respiratory tract disease caused by respiratory syncytial virus in pediatric patients (1999)
Herceptin (Trastuzumab, Humanized antibody directed against HER 2, i.e. human epidermal growth factor receptor 2)	Roche Registration	Treatment of metastatic breast cancer if tumor over-expresses HER2 protein (2000)
Indimacis 125 (Igovomab, Murine Mab fragment (Fab_2) directed against the tumor associated antigen CA 125)	CIS Bio	Diagnosis of ovarian adenocarcimona (1996)
Tecnemab KI (murine Mab fragments (Fab/Fab_2 mix) directed against HMW-MAA, i.e. high molecular weight meloma associated antigen)	Sorin	Diagnosis of cutaneous melanoma lesions (1996)
LeukoScan (Sulesomab, murine Mab fragment (Fab) directed against NCA 90, a surface granulocyte non-specific cross reacting antigen)	Immunomedics	Diagnostic imaging for infection/ inflammation in bone of patients with osteomyelitis (1997)
Humaspect (Votumumab, Human Mab directed against cytokeratin tumor associated antigen)	Organon Teknika	Detection of carcinoma of the colon or rectum (1998)
Mabthera (Rituximab, Chimaeric Mab directed against CD 20 surface antigen of B-lymphocytes)	Hoffmann La Roche	Non-Hodgkin's lymphoma (1998)
Mabcampath (EU) or Campath (USA); alemtuzumab; a humanized monoclonal antibody directed against CD 52 surface antigen of B-lymphocytes)	Millennium & ILEX	Chronic lymphocytic leukaemia (2001)
Therapeutic enzymes and additional products		
Cerezyme (rβ-glucocerebrosidase produced in *E. coli*. Differs from native human enzyme by one amino acid, arg 495 is substituted with a his, also has modified oligosaccharide component)	Genzyme	Treatment of Gaucher's disease (1997)
Fabrazyme (rh α-Galactosidase produced in CHO cells)	Genzyme	Fabry disease (α-galactosidase A deficiency) (2001)
Replagal (rh α-Galactosidase produced in a continuous human cell line)	TKT Europe	Fabry disease (α-galactosidase A deficiency) (2001)
Fasturtec (rasburicase; rUrate oxidase produces in *S. cerevisiae*)	Sanofi-Synthelabo	Hyperuricaemia (2001)
Osteogenic protein 1 (rhOsteogenic protein −1:BMP-7, produced in CHO cells	Howmedica (EU)	Treatment of non-union of tibia (2001)
Inductos (dibotermin alfa; rBone morphogenic protein-2 produced in CHO cells)	Genetics institute BV	Treatment of acute tibia fractures (2002)

[a] Notes: (a) Several products have been approved for multiple indications. Only the first indication for which it was approved is listed. (b) Products are listed by trade name. Abbreviations: r = recombinant; rh = recombinant human; CHO = Chinese hamster ovary; BHK = baby hamster kidney; Mab = monoclonal antibody; tPA = tissue plasminogen activator; hGH = human growth hormone; FSH = follicle stimulating hormone; TSH = Thyroid stimulating hormone; EPO = erythropoietin; GM-CSF = granulocyte-macrophage colony stimulating factor; IFN = interferon; IL = interleukin; HBsAg = Hepatitis B surface antigen; PDGF = platelet derived growth factor; and TNFR = tumor necrosis factor receptor.

committees, the committee for proprietary medicinal products and the committee for veterinary medicinal products. These are responsible for formulating the EMEA's scientific opinion on marketing applications relating to human and veterinary products, respectively. Marketing applications for all biotechnology-based medicines are evaluated through a 'centralized' system, which entails evaluation of the application dossier within a 210 day time frame. A scientific conclusion (decision) is reached by the committee, which is then conveyed to the European Commission (EC, Brussels). The commission has a maximum 90 days to consider the EMEA's scientific opinion and to grant (or refuse) a marketing authorization.

The European Commission has granted marketing licences to some 265 new pharmaceutical products since the introduction of the centralized approval process in 1995. Ninety-five of these were products of biotechnology. This represents 36% of all new drugs (i.e. new chemical entities and new biotech drugs) approved within this timeframe.

3. Products approved to date

All 88 biopharmaceutical products currently approved within the EU are protein-based. No gene therapy-based product has gained approval. The single antisense-based product approved in 1999 has subsequently been voluntarily withdrawn from the market by its sponsoring company. Of the proteins thus far approved hormones and cytokines represent the largest product categories (23 and 18 products respectively). Hormones approved include several recombinant human insulins, displaying both native and modified amino acid sequences (Table 1). In addition several recombinant gonadotrophins (follicle stimulating hormone, FSH; luteinizing hormone, LH; and human chorionic gonadotro-

phin, hCG) have been approved for the treatment of various forms of subfertility/infertility.

Cytokines approved include a range of recombinant haematopoietic factors, including multiple erythropoietin-based products used for the treatment of anaemia as well as a colony stimulating factor aimed at treating neutropenia. Additional approved cytokines include a range of recombinant interferon-based products, mainly used to treat cancer and various viral infections, most notably hepatitis B and C, and a recombinant tumour necrosis factor (TNF) used as an adjunct therapy in the treatment of some soft tissue cancers.

Blood-related approved therapeutic proteins include a range of recombinant blood coagulation factors used to treat haemophilia, recombinant thrombolytics and recombinant anticoagulants (Table 1). Additional product categories include a range of subunit vaccines containing at least one recombinant component (mainly hepatitis B surface antigen, HBsAg) and a variety of monoclonal antibody based products indicated for the treatment/detection of various cancers or the prevention of organ transplant rejection.

4. Trends in product approvals

Many of the initial therapeutic proteins approved for general medical use were simple replacement proteins (e.g. recombinant insulins and blood factors). Although some more recently approved products too are replacement proteins (e.g. recombinant human α-galactosidase used to treat Fabry disease), an increasing proportion of newly developed biopharmaceuticals are engineered. Protein engineering (site directed mutagenesis) entails the controlled alteration of a gene's nucleotide sequence, such that specific pre-determined alterations in the resultant polypeptide's amino acid sequence are introduced [5].

Although still a pursuit in its infancy, some notable progress has been made in elucidating the link between protein function and structure [6]. Protein engineering is increasingly used to tailor the functional attributes of commercially important proteins. It has been applied thus far to a number of therapeutic proteins in order to achieve various objectives, including: (a) alteration of the protein's

Table 2
Some biopharmaceuticals generated by protein engineering which have thus far gained approval for general mediel use within the EU[a]

Product type	Change(s) introduced to the structure of the native protein	Rationale for change
Chimaeric antibodies: Mabthera, Remicade and Simulect	Chimaeric antibodies consist of a mouse-derived variable region (which houses the antigen binding sites; the CDRs) and a constant region derived from a human antibody	Reduced immunogenicity when administered to man. Ability to activate human effector functions
Humanized antibodies: Synagis, Zenapax, Herceptin and Mabcampath	Humanized antibodies, consist of an antibody of human sequence into which CDRs from a murine monoclonal has been grafted.	Eliminated immunogenicity. Ability to activate human effector functions.
Modified insulins: Insulin lispro (sold under tradenames Humalog and Liprolog)	Identical to human insulin in amino acid sequence except for an inversion of the natural proline-lysine sequence on the B chain at positions 28 and 29	Generation of a faster acting insulin
Insulin aspart (sold under tradename NovoRapid)	Identical to human insulin in amino acid sequence except that proline found normally in position 28 of the B chain has been replaced by aspartic acid	Generation of a rapid acting insulin
Insulin glargine (sold under tradenames Lantus and Optisulin)	Differs from human insulin in that asparagine (A 21) is replaced by glycine and two arginines have been added to the B chain C-terminus	Generation of a long acting insulin
Modified tPA: Reteplase (sold under tradenames Ecokinase and Rapilysin)	Removal of 3 of the 5 domains characteristic of native tPA (the amino terminal finger domain, the EGF domain and the Kringle 1 domain)	Generation of a faster acting thrombolytic agent
Synthetic interferon: Infergen	Synthetic type 1 interferon, containing the most frequently observed amino acids in each corresponding position of several naturally occurring human IFN α subtypes	Displays higher antiviral, antiproliferative and NK cell activating activity as compared to most native IFN-αs
Modified blood factor VIII: (Tradename; ReFacto)	Differs from native human factor VIII in that its B domain has been deleted	Production of a lower molecular mass product which retains native factor VIII biological activity
Fusion proteins: Enbrel	Consisting of the extracellular ligand-binding portion of the human TNF receptor fused to the Fc portion of human IgG	Inhibits activity of TNF by binding it

[a] Additional information relating to specific products listed can be obtained from Table 1.

immunogenicity; (b) alteration of biological half life; (c) generation of faster/slower acting product; and (d) the generation of novel hybrid/synthetic therapeutic proteins (Table 2)

4.1. Engineered antibodies

Classical hybridoma technology facilitates the generation of essentially an unlimited supply of monospecific antibody, raised against virtually any antigen of interest [7]. The high degree of binding specificity exhibited by an antibody for the antigen against which it was raised underlines their use as analytical tools in the diagnostic and allied industries. A more recent application of monoclonal antibodies entails their use as in vivo diagnostic or therapeutic agents [8]. Antibodies raised against, for example, a tumour surface antigen should selectively bind to the surface of tumour cells. First generation monoclonal-based pharmaceutical products were unmodified murine antibodies/antibody fragments. As such these products themselves elicited the production of neutralizing antibodies when injected into humans (the so-called human anti-mouse antibody or 'HAMA' response). Additionally, these antibodies could not trigger effector functions such as the activation of complement in humans. Such drawbacks rendered disappointing the clinical efficacy of most such preparations.

Protein engineering provides a means of largely overcoming such difficulties. Chimaeric antibodies are generated by splicing the gene sequence coding for the mouse-derived 'variable' (V) antibody domains (which house the antigen binding sites; the Complementary determining regions (CDRs)) to a nucleotide sequence coding for the remainder (i.e. the constant or 'C' regions or domains) of a human antibody. The resultant hybrid antibody, while still recognizing the original antigen of interest, is significantly less immunogenic when administered to man. In addition, when compared to murine monoclonals, injected chimaeric products display a significantly extended circulatory half life (250 h as opposed to 40 h) and are also capable of mobilizing human immune system effector functions.

An alternative strategy entails an even more extensive engineering approach. The generation of humanized monoclonals involves identification of the short nucleotide sequences coding for the antigen binding sites – the CDRs- of the murine antibody displaying the required binding specificity. These sequences are then used to replace the CDR sequences in a human antibody gene. The resultant antibody is entirely human in nature, apart from its antigen binding sites. Predictably such humanized products are significantly less immunogenic than even chimaeric antibodies, and they display serum half lives indistinguishable from native human antibodies. Over the last number of years a number of both chimaeric and humanized antibodies have been approved for general medical use (Tables 1 and 2).

4.2. Engineered insulins

A number of both fast and slow acting engineered insulin products have also been approved for general medical use over the past few years (Table 2). When stored at therapeutic dose concentrations individual insulin molecules interact with each other, forming oligomers (mainly hexamers). Upon its sc or im administration, the entry of insulin into the bloodstream is delayed by the requirement for initial deoligomerization. A practical consequence is the requirement to inject the insulin preparation up to 1 h before eating which can be inconvenient, and potentially dangerous should the recipient subsequently alter the planned mealtime.

Experimental investigations reveal that various alterations in amino acid sequence towards the C terminus of insulin's B chain decreases the propensity of individual hormone molecules to self-associate [9]. A few such altered insulins retain normal biological activity and remain non-immunogenic in humans, while being capable of rapid entry into the bloodstream [10]. These engineered products can be administered at mealtime and several have been approved for general medical use since the late 1990s (Tables 1 and 2). Insulin glargine (international non-proprietary name; sold under the trade names lantus and opsulin; Table 2) is a recently approved engineered human insulin analogue displaying a significantly increased duration of activity. The sequence changes made increases the insulin molecule's pI (isoelectric point; the pH at which it displays no net charge, and hence is least soluble) from a value of 5.4 to one closer to 7.0. When formulated at an acidic pH this product remains soluble. Upon its administration physiological pH triggers the formation of insulin microprecipitates at the site of injection from which individual insulin molecules enters general circulation only very slowly.

4.3. Additional engineered products

The tissue plasminogen activator (tPA) based products 'Ecokinase', 'Rapilysin' and 'Tenecteplase' represent yet another group of engineered, second generation approved therapeutic proteins. Recombinant tPA has proven effective in the treatment of myocardial infarction. However, the native protein displays a short plasma half life (3 min), necessitating its infusion into patients for periods of up to 90 min. The sequence alterations introduced (Table 2) significantly extends the molecule's half life, facilitating its administration by a single iv injection.

Enbrel (trade name) represents a different class of engineered therapeutic protein. In this case molecular engineering has been utilized to generate a truly novel, hybrid protein. This consists of the extracellular domain of the tumour necrosis factor receptor genetically fused to the constant portion of human IgG (Table 2).

4.4. Pegylated proteins

An alternative form of protein engineering entails the covalent attachment of polymers (mainly polyethylene glycol; PEG) to the protein's backbone [11]. Pegylation generally increases the plasma half-life of therapeutic proteins. Native interferon-αs, for example, typically display plasma half lives of the order of 4 h. Pegylation generally increases the observed value by anything up to 20 h. As a result a number of second-generation (i.e. pegylated) interferon products have recently come on the market (Table 1).

5. Future trends

The biopharmaceutial sector, now well established, continues to grow rapidly. While European figures are difficult to locate, the American association of pharmaceutical researchers and manufacturers (PhRMA) estimate that there are currently some 371 biotechnology medicines in development [12]. Well in excess of 300 of these are protein based, with recombinant vaccines and monoclonal/engineered antibodies representing the two most significant product categories. The most common target indication is cancer. Over the coming decade, therefore, in the region of a dozen new therapeutic proteins should, on average, gain regulatory approval each year.

Currently virtually all approved recombinant proteins are expressed in engineered *Escherichia coli* cells, *Saccharomycese cerevisiae* or animal cell lines (almost exclusively in Chinese hamster ovary or baby hamster kidney cell lines). While these will remain the foremost new product production cell lines, it is likely that some proteins produced in alternative systems will gain regulatory approval in the not too distant future. Transgenic animal and plant systems are particularly noteworthy in this regard. The production of pharmaceutical proteins in the milk of transgenic animals may yet prove to be technically and economically attractive [13,14]. At least two companies (GTC biotherapeutics; formerly genzyme transgenics, and PPL therapeutics) are sponsoring the clinical evaluation of a range of recombinant proteins produced by such means. These include α_1-antitrypsin, antithrombin and a range of engineered antibodies. The production of therapeutic proteins in transgenic plants also continues to generate interest [15,16]. Expression of oral vaccines in edible plants/fruit such as tomatoes and bananas is gaining most attention in this regard.

5.1. Innovations in protein delivery

To date all approved therapeutic proteins targeted to the bloodstream are administered parenterally. Although a non-issue in the context of once off administered product, alternative (non-parenteral) modes of delivery would be preferable in the context of frequently administered products (e.g. insulin or blood factors). Non-parenteral administration would be more convenient, less invasive and likely to achieve better patient compliance. Oral delivery systems, not unsurprisingly, remain problematic in the case of proteins. Alternative systems, in particular nasal and pulmonary routes, show more promise [17–19]. Uptake rates across the nasal epithelia are dependent upon protein molecular mass. Peptides and small polypeptides often cross with relative ease, although polypeptides of molecular mass greater than 10 kDa (i.e. most biopharmaceuticals) generally cannot do so without concurrent administration of penetration enhancers.

Pulmonary delivery currently represents the most promising alternative to parenteral delivery for proteins. Many macromolecules are absorbed from the deep lung surprisingly well. Protein bioavailabilities (relative to sc administration) of at least several percent and (in rare cases) up to almost 50% have been recorded. Although no such product had been approved to date, an inhalable insulin preparation (tradename Exubera) remains a leading candidate. Currently in phase III clinical trials, additional critical safety studies are currently underway.

5.2. Nucleic acid-based products

Despite initial enthusiasm, it remains a fact that no gene therapy-based product has thus far been approved (in either the EU or USA), and that only a single antisense product has gained approval. The antisense drug (tradename vitravene) is a 21 nucleotide phosphorothiolate-based product, which displays sequence complementary to certain human cytomegalovirus mRNA transcripts. It therefore inhibits viral replication via an antisense mechanism, and is used to treat cytomegalovirus retinitis in AIDS patients. Approved initially in the USA in 1998 and the following year in the EU, the product was subsequently withdrawn from the EU market, for commercial rather than technical/safety reasons. Of the 371 biotechnology medicines in development according to PhRMA, only nine are antisense-based and 16 are gene therapy-based.

The failure of nucleic acid-based products to thus far meet original expectations in no way reflects a flaw in the concepts of gene therapy or antisense technology. These technologies retain the potential to revolutionize medical practice. Their full benefit however, will only accrue when several associated technical difficulties are overcome. These include issues pertaining to gene/nucleotide delivery, stability and regulation of expression. Clinical trials to date have also reported a disappointing lack of efficacy and some have raised safety concerns [20–23].

5.3. Genomics and proteomics

Genomics refers to the systematic study of the entire genome of an organism. It entails sequencing the entire cellular DNA complement and the assignment of exact positions within the genome to the various genes and non-coding regions [24]. To date the sequence of the entire

genome of man and several higher animal species, various plants and almost 70 microorganisms have been completed/almost completed. Proteomics, in contrast, focuses upon the study of the entire expressed protein complement of the cell (the proteome). Unlike the genome, which is essentially static in nature, the proteome is not static, with changes in cellular conditions triggering changes in cellular protein expression profiles [25].

Genomic and proteomics investigations continue to generate huge quantities of raw sequence data, and amongst all this protein/gene data no doubt lie many potential new biopharmaceuticals/biopharmaceutical targets. The focus of study in this area now has turned towards assignment of biological function to these genes/gene products (functional genomics). All the major pharmaceutical companies have major interests in this area, and it is only a matter of time before such research generates the next generation of potential biopharmaceuticals/targets for clinical evaluation.

6. Concluding remarks

Products of modern pharmaceutical biotechnology now represent a very significant fraction of the total pharmaceutical market. Technical innovations, particularly in molecular biology, facilitated the rapid development not only of simple replacement proteins, but also engineered protein pharmaceuticals. Gene therapy and antisense technology remain to fulfil their therapeutic promise, but when the technical hurdles currently impeding their utilization are overcome, they promise to revolutionize many aspects of medical practice. The advent of genomics, proteomics and related disciplines will also accelerate the future identification of putative biopharmaceuticals and in particular biopharmaceutical (and other drug) targets. As such, biopharmaceutical based products will likely represent an even greater proportion of the total pharmaceutical portfolio in the future.

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