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REVIEW ARTICLE

Insulin Biosimilars: The Impact on Rapid-Acting Analogue-Based Therapy

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Abstract The impending expiration of patent protection for recombinant insulins provides the opportunity to introduce cost-saving copies, named biosimilars, onto the market. Although there is broad experience in the production and characterisation of insulins, the development of copies is still a challenge. In this paper, the main features of insulins and the EU regulatory framework for their biosimilar products are reviewed. The main focus is on rapid-acting insulin analogues (Humalog®; Novolog®/NovoRapid®; Apidra®). Since they differ by one or two amino acids in chain B, production of one biosimilar for all three drug products is not feasible. However, from post-marketing-collected clinical data, rapid-acting insulin analogues seem to have similar therapeutic efficacy. It is reasonable to suppose that, for prescription to treatment-naïve patients, the cheaper biosimilar would be the preferred choice of physicians, either spontaneously or induced by health insurance. Therefore, its introduction will affect the market share of all the other rapid-acting insulin analogues.

Key Points

The first biosimilar of an insulin-based product has been recently approved in the EU.

Three rapid-acting analogues are available on the EU market; they have slightly different pharmacokinetic parameters that don't seem to influence the safety and efficacy profile.

Market availability of a rapid-acting analogue biosimilar will have an impact on the prescription of all the products in the same class and their use in clinical practice.

1 Introduction

Patent protection for recombinant insulins and their analogues have expired or are about to expire, providing the opportunity to develop low-priced copies (Table 1). Price reduction is possible, since research and the studies needed to demonstrate the product's efficacy and safety have already been performed by the Marketing Authorisation holder of the originator. Copies of biological medicinal products are named 'biosimilars' as they contain an active substance which is similar to the one contained in an authorised biological medicine. However, since recombinant proteins are obtained by complex biotechnological processes, molecules produced by different manufacturers may present different structural features that can lead to distinct pharmacokinetic (PK) and pharmacodynamic (PD) profiles [1]. As a consequence, regulatory agencies use a different approach for biosimilars from the one applied to

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Table 1 Insulin medicinal products authorised in the EU with a centralised procedure

Insulin class	INN	Proprietary name	Manufacturer of the biological active substance responsible for batch release	MA date
Rapid-acting insulin	Insulin aspart	NovoRapid	Novo Nordisk A/S	07/09/1999
Rapid-acting insulin analogue	Insulin glulisine	Apidra	Sanofi-Aventis Deutschland GmbH	27/09/2004
Rapid-acting insulin analogue	Insulin lispro	Humalog	Eli Lilly	30/04/1996
Rapid-acting insulin analogue	Insulin lispro	Liprolog	Eli Lilly	01/08/2001
Short-acting insulin (neutral insulin solution)	Insulin human (rDNA)	Actrapid	Novo Nordisk A/S	07/10/2002
Short-acting insulin (neutral insulin solution)	Insulin human (rDNA)	Insulin Human Winthrop Rapid	Sanofi-Aventis Deutschland GmbH	17/01/2007
Short-acting insulin (neutral insulin solution)	Insulin human (rDNA)	Insuman Rapid	Sanofi-Aventis Deutschland GmbH	21/02/1997
Intermediate-acting insulin (insulin + protamine isophane)	Insulin human (rDNA)	Insulatard	Novo Nordisk A/S	07/10/2002
Intermediate-acting insulin (isophane insulin suspension)	Insulin human (rDNA)	Insuman Basal	Sanofi-Aventis Deutschland GmbH	21/02/1997
Intermediate-acting insulin (insulin + protamine isophane)	Insulin human (rDNA)	Insulin Human Winthrop Basal	Sanofi-Aventis Deutschland GmbH	17/01/2007
Intermediate-acting insulin (insulin protamine suspension)	Insulin lispro	Liprolog Basal	Eli Lilly	01/08/2001
Intermediate-acting insulin (insulin isophane [NPH] suspension)	Insulin human (rDNA)	Protaphane	Novo Nordisk A/S	07/10/2002
Mix of short-acting (soluble) insulin and intermediate-acting insulin (isophane)	Insulin human (rDNA)	Actraphane	Novo Nordisk A/S	07/10/2002
Mix of short-acting (soluble) insulin and intermediate-acting insulin (isophane)	Insulin human (rDNA)	Insuman Comb	Sanofi-Aventis Deutschland GmbH	21/02/1997
Mix of short-acting (soluble) insulin and intermediate-acting insulin (isophane)	Insulin human (rDNA)	Insulin Human Winthrop Comb	Sanofi-Aventis Deutschland GmbH	17/01/2007
Mix of short-acting (soluble) insulin and intermediate-acting insulin (isophane)	Insulin human (rDNA)	Mixtard	Novo Nordisk A/S	07/10/2002
Mix of rapid insulin lispro and intermediate-acting insulin lispro-protamine	Insulin lispro	Liprolog Mix	Eli Lilly	01/08/2001
Mix of rapid-acting insulin aspart and intermediate-acting protamine-crystallised insulin aspart	Insulin aspart	NovoMix	Novo Nordisk A/S	01/08/2000
Long-acting insulin analogue	Insulin glargine	Lantus	Sanofi-Aventis Deutschland GmbH	09/06/2000
Long-acting insulin analogue	Insulin glargine	Optisulin	Sanofi-Aventis Deutschland GmbH	27/06/2000
Long-acting insulin analogue	Insulin detemir	Levemir	Sanofi-Aventis Deutschland GmbH	01/06/2004
Long-acting insulin	Insulin degludec	Tresiba	Novo Nordisk A/S	21/01/2013
Mix of long-acting insulin (degludec) and rapid-acting insulin aspart	Insulin degludec/ insulin aspart	Ryzodeg	Novo Nordisk A/S	21/01/2013

http://www.ema.europa.eu/ema/index.jsp?curl=pages%2Fmedicines%2Flanding%2Fepar_search.jsp&mid=WC0b01ac058001d124&searchTab=&alreadyLoaded=true&isNewQuery=true&status=Authorised&status=Withdrawn&status=Suspended&status=Refused&keyword=Enter+keywords&searchType=name&taxonomyPath=Diseases.Hormonal+diseases&treeNumber=&currentCategory=Diabetes+Mellitus&searchGenericType=generics

INN International Non Proprietary Name, *MA* Marketing Authorisation, *NPH* neutral protamine Hagedorn

generic products. Indeed, non-clinical and clinical data cannot be replaced by bioavailability assays as is the case with generics. Instead, comparative non-clinical and clinical studies should be performed, and they are defined case by case according to the guidelines of the specific biosimilar class (epoetins, insulins, monoclonal antibodies, low-molecular-weight heparins [LMWHs]). As a result, the Marketing Authorisation Application (MAA) for a copy of

a biological product relies on the demonstration of similarity of the biosimilar and reference product in term of quality, efficacy and safety through the so-called 'comparability exercise' [2].

At present, several insulin-based products are available on the market. Insulin products authorised by the European Medicines Agency (EMA) with a centralised procedure are listed in Table 1, along with brand products, such as Humulin® and Novolin®, authorised with the multistate procedure before Regulation (EEC) 2309/1993 came into force.

Until now, all products have been approved with a full dossier as originators or as products developed in co-marketing.

On July 2013, Eli Lilly & Co. submitted to the EMA an application for an insulin glargine-containing biosimilar that received a positive opinion from the Committee for Medicinal Products for Human Use (CHMP) on 26 June 2014 [3], and was authorised in September 2014. In May 2013, Sanofi announced the start of the clinical trials for Humalog® and NovoLog® biosimilars. This seems to indicate that other insulin biosimilars are expected to hit the European market in the next few years.

In this paper, the main features of insulin and insulin analogues are discussed with particular attention to rapid-acting analogues, namely insulin lispro (Humalog®; Eli Lilly), insulin aspart (Novolog®/NovoRapid®; Novo Nordisk) and insulin glulisine (Apidra®; Sanofi), taking into account the regulatory framework in force in the EU for the development of biosimilar products. On the basis of this analysis, the possible impact of the introduction to market of such copies on clinical practice is discussed.

2 Insulin Classes: Production and Critical Aspects

Human insulin is a non-glycosylated protein of 51 amino acids composed of two polypeptide chains (A and B) linked by disulfide bridges. This structure has represented the basis of diabetes mellitus treatment for a long time, but efforts have been made to develop products with improved PK profiles for better control of blood glucose levels [4]. The advent of DNA recombinant technology made it possible to make slight modifications to the insulin amino acid sequence, leading to the development of insulin analogues with more predictable absorption profiles [5].

Insulin and insulin analogues are divided on the basis of their PK profile in three main classes: fast-acting, intermediate-acting and long-acting insulins [6].

Fast-acting insulins are rapidly absorbed in the bloodstream and are essentially used to control postprandial glycaemia. They include rapid-acting insulin analogues (insulin aspart, insulin lispro, insulin glulisine) and short-acting insulin (regular human insulin). Rapid-acting insulin analogues have been developed by inserting bulky or charged amino acids (one or two modifications) at a specific point of human insulin chain B in order to promote repulsion between monomers, thus resulting in a faster action [7]. Indeed, rapid-acting analogues are absorbed within 30 min after subcutaneous injection, so they can be administered immediately before a meal. However, their action is shorter and they have to be combined with regular or long-acting insulins [6].

Intermediate-acting insulins, such as neutral protamine Hagedorn (NPH), have an onset of action ranging between 2 and 4 h and require approximately 6–7 h to reach peak concentration, with a duration of action of 12–16 h. Hypoglycaemic episodes may occur between meals and at night, so twice-a-day administration is thus required to minimise the daily excursions of insulin levels. Moreover, its action is often variable [8].

Long-acting insulins (insulin glargine and insulin detemir) are analogues with improved amino acid sequences specifically synthesised to have a constant and slow release of insulin over a 24-h period, allowing once-a-day dosing for patients [9]. In the case of insulin detemir, the slow release of the insulin analogue into the circulation is followed by the binding (98 %) to serum albumin, thus resulting in a buffering effect that limits PD variability [8]. Recently, two new long-acting analogues, insulin degludec and a pegylated insulin lispro, have been developed. Insulin degludec has already been authorised (Table 1), whereas pegylated insulin lispro is in clinical trials [10].

Finally, premixed insulin types are available to simplify dosing and for use in people using more than one type of insulin (mainly patients with type 2 diabetes). These formulations combine an intermediate-acting insulin (NPH) and a regular insulin or a rapid-acting insulin and a longer-acting insulin in order to reach a proper control of glycaemia at meal times as well as spanning the day. Typical examples are Humalog® mix and Novolog® mix (Table 2).

Insulin was the first protein synthesised by means of a biotechnological process and nowadays all insulin products on the market are manufactured by DNA recombinant technology.

The synthesis of a biotechnological protein is a highly complex process, involving 20–30 critical operations. Variability of the starting material as well as any changes in processing can drastically affect the quality of the final compound. Generally speaking, insulin synthesis relies on the insertion of the desired nucleotide sequence coding for the insulin precursor, pro-insulin, in a plasmid vector and its transfection into host cells (*Escherichia coli* or *Saccharomyces cerevisiae*) which express the protein precursor [11, 12].

After fermentation and recovery of pro-insulin, the precursor undergoes in vitro proteolytic cleavage yielding insulin, which is then purified by high-resolution chromatographic steps [7]. The manufacturing steps slightly change depending on the recombinant organism used. In *E. coli*, the fusion protein is released in inclusion bodies that have to be degraded to recover the insulin precursor, which has to be purified and folded before being cleaved; in yeast-based systems, instead, proteins are released already folded in the medium [12]. This implies that the subsequent steps of extraction and purification from inclusion bodies are only required when bacteria are used as expression vectors.

It is clear that in such a bioengineered process, each parameter has to be strictly monitored. Indeed, any variation in cell lines, media components, expression systems,

Table 2 Main features of rapid-acting insulin analogues [17–19]

	Insulin lispro	Insulin aspart	Insulin glulisine
Brand marker	Humalog (Ely Lilly)	Novorapid (Novo Nordisk)	Apidra (Aventis)
Indications	Treatment of diabetes mellitus and initial stabilisation of diabetes	Treatment of diabetes mellitus	Treatment of diabetes mellitus
Host organism	*Escherichia coli*	*Saccharomyces cerevisiae*	*E. coli*
Onset	15–30 min	10–20 min	10–15 min
Peak	30 min–2.5 h	1–3 h	1–1.5 h
Duration of action	3–6.5 h	3–5 h	3–5 h
T_{max}	30–70 min	30–40 min	55 min
$T_{½}$	26–52 min	81 min	42 min
Meal timing	Within 15 min before or immediately after meals	5–10 min before meals	Within 15 min before or within 20 min after starting a meal
Mixing with insulins	Can mix with NPH (Humulin N) Can mix with ultralente Do not mix with glargine	Can mix with NPH No data on mixing with regular, lente, or ultralente Do not mix with glargine	Can mix with NPH only
Pharmaceutical forms	Injection solution, cartridge, pens and pre-filled pens (KwickPen)	Injection solution, cartridge, pre-filled pen (Flexpen)	Injection solution (vials), cartridge, pre-filled pen (OptiSet, SoloStar)
Pre-mixed insulin available	Humalog mix 25/75: 25 % lispro suspension + 75 % lispro protamine Humalog mix 50/50: 50 % lispro suspension + 50 % lispro protamine	NovoLog® Mix 70/30: insulin aspart protamine suspension/insulin aspart	
Special populations	Pregnancy: studies on large no. of subjects Lactation: any specific study Children: studies on 61 patients 3–11 years old and 481 patients 9–18 years old. No differences with respect to adults Elderly: no specific studies but it can be used	Elderly (≥65 years old) Children: no studies under 2 years old Renal or hepatic impairment: may reduce the patient's insulin requirements	Elderly: limited PK data Children: insufficient data for children under 6 years old Hepatic impairment: PK not investigated Pregnancy: not enough data
Undesirable effects	Hypoglycaemia (very common) Local allergy Sistemic allergy (rare) Lipodystrophy (uncommon)	Urticaria, rash, eruptions (uncommon) Anaphylactic reactions (very rare) Hypoglycaemia (very common) Peripheral neuropathy (painful neuropathy) (rare) Refraction disorders (uncommon) Diabetic retinopathy (uncommon) Lipodystrophy (uncommon) Injection site reactions (uncommon) Oedema (uncommon)	Hypoglycaemia (very common) Hyperglycaemia (unknown) Injection site reactions Local hypersensitivity reactions Lipodystrophy Systemic hypersensitivity reactions

NPH neutral protamine Hagedorn, *PK* pharmacokinetics

bioreactor conditions as well as purification steps, may lead to products with varied quality attributes. For instance, different process-related impurities, even in small amounts, may elicit an immune response when the injectable medicinal product is finally administered. Moreover, the containers/closures (vials and syringes) used to protect dosage forms from the environment may strongly impact product performance and immunogenicity. The interaction between the dosage form and the container surface may also lead to changes in biological activity and cause protein aggregation, thus affecting the stability of the protein [13].

3 The Case of the Three Rapid-Acting Analogues

Insulins lispro, aspart and glulisine are individual drugs, characterised by the presence of modified amino acid residues in specific positions on the human insulin chain B (Fig. 1). Although the three analogues are all produced by DNA recombinant processes, Novo Nordisk chose to use *S. cerevisiae* instead of *E. coli* as host cells for insulin synthesis. The use of a different host organism involves a different manufacturing process (as mentioned above) and may significantly affect the characteristics of the protein, leading, for example, to an immunogenic response.

Since the three analogues have been independently authorised, few studies directly comparing these medicaments are available in the scientific literature. In clinical trials aimed at comparing the PK/PD activity of these analogues, slight variations in PK parameters were noted. However, no relevant differences in terms of hypoglycaemic effect and safety profile were found [14]. Moreover, the commercial formulations showed comparable insulin time-exposure and time-action profiles in a double-blind, six-way, crossover, euglycaemic glucose clamp study performed in 14 healthy volunteers, by injecting each analogue subcutaneously [15].

Clinical evidence, even if limited, may lead to the consideration that rapid-acting insulin analogues may be considered therapeutical equivalent in the treatment of normal populations of patients with type 1 and type 2 diabetes. However, a real three-way crossover clinical trial carried out in a significant number of subjects is still missing and the three analogues actually presented different features in the pre-marketing studies, as described in Table 2 [16–18].

Insulin glulisine shows a faster onset of action with respect to insulins aspart and lispro, probably due to the zinc-free formulation. Indeed, insulin lispro and insulin aspart preparations contain zinc to promote hexamer formation, whereas insulin glulisine is stabilised with polysorbate 20. The zinc may slow down the disassociation into monomers, delaying the biological effect. In contrast, insulin glulisine can be rapidly absorbed after subcutaneous administration [10].

In addition, rapid-acting insulin analogues are not identically indicated for the treatment of special populations. For instance, Apidra® has not been properly studied in pregnant women or in patients with reduced hepatic function. Moreover, there aren't enough data on the use of Apidra® in children younger than 6 years old. On the other hand, the absorption rate of NovoRapid® is decreased in patients with liver failure.

The adverse effects reported in the Summary of Product Characteristics (SPC) differ from one analogue to another. However, in all cases, hypoglycaemia is the most common adverse event. For Apidra®, hyperglycaemia cases were also reported but they were mainly due to handling errors or pump system failures [17].

As stated above, rapid-acting insulin analogues have to be associated with regular or long-acting insulin for proper control of glucose level during the whole day. In this context, the possibility of mixing and the existence of premixed insulins play a key role for the compliance of patients. Apidra® can be mixed only with NPH insulin and premixed products containing Apidra® are not available on the market.

Finally, each pharmaceutical company has put on the market its own delivery devices and, if pens are considered,

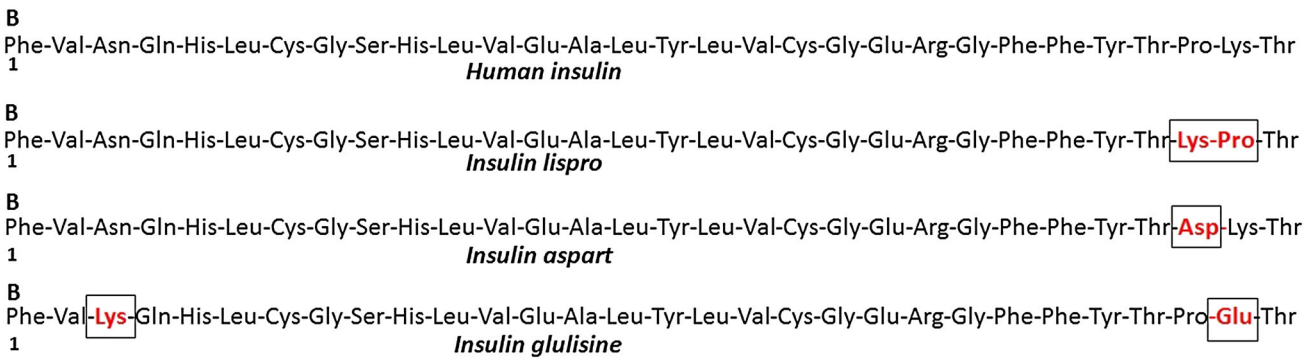

Fig. 1 Modification of the amino acid sequence of chain B of human insulin in the three rapid-acting insulins

not all the needles are compatible with pens produced by various firms.

4 Regulatory Dispositions for Biosimilar Insulins

In 2013 and 2014, the EMA published revisions to the guidelines on quality clinical and non-clinical issues for similar biological medicinal products containing biotechnology-derived proteins as the active substance [19, 20]. Moreover, in the same period, the specific guidelines for non-clinical and clinical development of biosimilars containing recombinant human insulin and insulin analogues were also revised [21, 22].

According to these guidelines, in the MAA dossier for an insulin biosimilar the applicant has to fully characterise the manufacturing process, the active substance, including the structures, the variants and the isoforms, as well as each component included in the final formulation. The biosimilar must not be identical to the reference product in terms of formulation or excipients but the changes made should be appropriately justified and they do not have to impact on the safety of the final product [23].

According to the EMA, module 3 of the MAA dossier of a biosimilar product should include, together with a full quality section, the results of the 'comparability exercise'. This is carried out by performing appropriate and validated physicochemical comparability in vitro tests in comparison with several, well identified batches of a reference product approved in the European Union (EU). Since 2013, the EMA also accepts as a reference drugs approved in a non-European country with similar regulatory standards and as long as certain data is provided [23].

As far as the comparability exercise is concerned, its scope is to demonstrate that the biosimilar and reference product are really similar for the quality features that may affect safety and efficacy profiles, whereas minor differences, if justified, are accepted. Therefore, the selected analytical methods and the chosen criteria have to be suitable to highlight eventual differences and not only the similarities. The quality comparability exercise involves the physicochemical, biological and immunochemical comparison between biosimilar and reference product as well as a deep analysis of the purity and impurity profiles, which has significant meaning, as already discussed. Process-related impurities are expected to be qualitatively different but their immunogenicity has to be assessed [20].

Another concern is the device. Each device (cartridges, pens, syringes) has to be tested with each formulation and dosage form, therefore for the biosimilar preparation the compatibility and the choice of the device have to be demonstrated [11]. One of the numerous criticisms raised by the EMA on the first MAA presented by Marvel in 2008 concerned the lack of information on the device intended for the cartridge formulation presented, as well as the lack of results of suitability tests on these devices [24]. In fact, adverse effects can be ascribed to an improper delivery of the insulin dose; thus, the accuracy and reproducibility of the dosage has to be guaranteed when the patient replaces the brand with the biosimilar product [12].

The non-clinical section includes in vitro affinity and binding assays to the IGF-1 receptor (including on–off kinetic) and determination of intrinsic biological activity. Receptor autophosphorylation, metabolic and mitogenic activity should be investigated during the determination of biological activity.

The predictability of the therapeutic response to a biosimilar with respect to the reference product is assessed in vivo by means of a double-blind euglycaemic clamp study by administration of a single subcutaneous dose in patients with type 1 diabetes or, eventually, in healthy volunteers after suppression of the endogen insulin.

With the last revisions of the guidelines, the EMA went deeply into the description of the clamp studies, the criteria of inclusion of the population enrolled in the clamp test and the standard conditions to be maintained during the study [21, 22]. In particular, in the last draft, all the PK and PD endpoints were detailed taking into account the peculiarity of each class of insulin preparations: rapid and short-acting, intermediate-acting and long-acting insulins. As an example, the last class have a very flat PK profile; therefore, the determination of maximum concentration and time to maximum concentration may not be meaningful, thus the EMA issued specific ad hoc guidelines for this class of products [22].

When different types of preparations (rapid, intermediate and long-acting insulins) are developed by the same biosimilar manufacturer, comparability PK and PD assays should be performed for the soluble insulin preparations, whereas only the similarity of PK profiles of the other insulin preparations with their respective reference medicinal products should be demonstrated.

Immunogenicity tests carried out in patients with type 1 diabetes for a period of at least 12 months, including 6 months of comparative phase (with titration of anti-drug antibody), are also recommended.

In case a biosimilar manufacturer develops different preparations, only a single safety study is usually required, except when the formulation contains excipients for which limited experience exists. In that case, a separate safety/immunogenicity study should be carried out.

Finally, a pharmacovigilance plan for the biosimilar product has to be presented with the MAA dossier [22].

A cause of concern is the carcinogenic hazard discovered for insulin, mainly for insulin analogues. Indeed insulin, by binding to the IGF-1R, along with the primary

function of lowering the blood level of glucose, exerts a mitogenic effect. This activity may be increased for the insulin analogues obtained by modification of the native insulin. As an example, insulin glargine was found to induce proliferation of breast and colon cell lines and human lung fibroblasts in vitro [25]. In 2009, CHMP requested to the proprietary company to provide clinical data to exclude the connection between insulin glargine and cancer. Overall, three population-based studies were carried out, two cohort and one case–control, and the data collected did not provide evidence of an increased risk of cancer with insulin glargine [26]. However, the carcinogenesis of insulin analogues may be a public health hazard, since these medicaments are administered over a lifetime. For this reason, the Committee for Proprietary Medicinal Products (CPMP) of the EMA published in 2011 a guidance on the approach to follow for the assessment of the carcinogenic potential of new insulin analogues [27]. Moreover, in the revised guidelines on non-clinical and clinical development of insulin and insulin analogue biosimilars, the evaluation of mitogenic potential in cells expressing the IGF-1 receptor has been introduced as part of the comparability exercise [22]. However, a full battery of carcinogenic studies is not necessary for biosimilar products with the same structure and biological activity as that of reference compounds. The experience gained on the therapeutic use of insulin together with the univocal clinical indication and the well defined class of patients, allowed the regulatory agency to issue a more detailed guideline with respect to other classes of biosimilar products such as LMWHs, for which the originator products themselves have a high degree of structural variability [28]. These guidelines do not overcome the complexity of development of copies of insulin products but, at least, help pharmaceutical companies to plan and carry out the production and characterisation of their biosimilar medicines.

5 Discussion

The impending expiration of patent protection for various insulin products provides the opportunity to introduce cost-saving copies onto the market.

The development of a biosimilar of a biotechnological protein is complex since the quality, safety and efficacy profiles of the product strongly depends on process variables, and each deviation from the original method may influence structural properties such as folding of the final protein and consequently its biological activity.

In order to facilitate the introduction onto the market of less expensive copies of insulin-based products, the EMA has issued and updated specific guidelines which dictate the requirements for a product claiming to be ‘similar’ to one already authorised by CHMP (through a centralised procedure). The precautionary approach adopted by the EMA for biosimilars is exemplified by the fact that, in Europe, the first Marketing Authorisation for a copy of a specific insulin was only issued in September 2014, whereas many applications had been submitted and various insulin biosimilars were already on the market in other countries, such as China, India, Pakistan, Peru, Thailand, and Mexico [29]. The difficulty of developing a valid copy of an insulin product has been proved by the Marvel experience. Indeed, the company was compelled to withdraw its MAA for the biosimilar of Humulin, twice. In 2008, several concerns about the quality of the non-clinical and clinical data provided were raised; in 2012, the draft of the revision of the guideline on human insulin and insulin analogues was published by the EMA and the applicant didn’t comply with the updated requirements for the PK/PD clamp studies [24, 30]. In fact, the guidelines concerning the authorisation of biosimilar products containing insulin as an active substance have become gradually more detailed and exhaustive.

However, it is important to underline that insulin is a non-glycosylated protein with a defined structure that will not change in biosimilar medicaments. Moreover, there is a wealth of experience in the production of insulin and insulin analogues and physicochemical and biological methods are available for the characterisation of its structure and activity [22]. These considerations don’t remove all the concerns connected to the development of insulin copies, but make the biosimilar approach and the evaluation of insulin copies less controversial compared with more complex classes of biological products such as LMWHs and erythropoietin [2, 28], as clearly stressed in the last revision of the guideline on biosimilar medicinal products [23].

As far as the rapid-acting analogues are concerned, according to the current guideline, the development of a unique biosimilar to all three brand products is not feasible since, in that case, the copy and the reference protein should share the same amino acid sequence [23]. However, the clinical data available in the literature highlight a substantial similarity of the three insulins in terms of PK and PD profiles determined in post-marketing studies [31, 32]. Despite the fact that some PK parameters are not perfectly superimposable, the overall clinical trials reveal that the three compounds have almost superimposable clinical efficacy and safety profiles [14]. That, and the fact that they have the same therapeutic indications and the same duration of action, discloses the possibility of applying the principle of therapeutic equivalence to rapid-acting analogues, which could induce health insurance companies to consider one analogue’s biosimilar as therapeutically equivalent with the other two products of

the same class. This may be a cause for concern, since it may strongly affect medical practice, as clearly evidenced in the position statement published last year by the Italian association of diabetology (SID) [33]. It may be plausible that the applicant submits an MAA for a product claimed to be biosimilar to one of the three analogues, providing the possibility of demonstrating therapeutic similarity to the other two products in suitable clinical comparative studies carried out after authorisation. It should be also underlined that differences in terms of devices and dosage forms developed as well as indications in special and normal populations will persist.

Moreover, unlike generics, automatic substitution between reference and biosimilar products is not expected. In fact, as clearly stated in the SPC of all insulin-containing medicines, every change may require an adjustment of the dosage [34] and therefore has to be evaluated by healthcare professionals. However, it should be considered that in diabetic patients, blood glucose levels can be affected by many factors including diet, exercise, stress, and illness. Therefore, dosage adjustment is constantly required, independently from the switch from one brand of insulin to another. In the first years of commercialisation of a biosimilar, the switching has to be carefully evaluated for patients already receiving treatment, while no problem arises for treatment-naïve patients. In the latter case, the physician may decide, or be compelled by the health insurance company, to prescribe directly the cost-saving product affecting the market share of all the other rapid-acting insulin analogues.

Finally, it should be mentioned that EMA does not recommend the interchangeability between copy and brand product but refers to the policies of each Member State [23]. Therefore, the rigorous policy that rules the Marketing Authorisation grant for a biosimilar is not enough without a suitable education plan for healthcare professionals.

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Author contributions P. Minghetti conceived the paper; S. Franzè and F. Cilurzo wrote the manuscript. P. Minghetti critically revised for intellectual content. All authors approved the final version of the manuscript.

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