Formulation Techniques Used for Insulin Analogs Preparation, Types and Action: A review article

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Abstract

Insulin is a peptide hormone released as pro-insulin from pancreatic β-cell consist of A and B chain and C-peptide, insulin formed after cleavage of C-peptide from pro-insulin. In response to meal, exaggerated insulin exocytosis occur to utilize glucose while basal insulin exocytosis maintain euglycemia (normal blood concentration of glucose). Insulin's intrinsic ability to quick association into dimers and advanced order states is an important property. In the presence of zinc, the ability to shape discrete hexamers has been used to create medicinally valuable insulin formulations. A conformational change that favors chemical stability (from T6 to R6) can obtained by binding of phenolic excipients to unique sites on insulin hexamers and this will lessen the aggregation of high molecular weight polymer. Another important property of insulin that can be exploit during therapeutic formulation is the insulin's isoelectric point (PI) in the denatured state is 5.3. Fibrillary aggregation can be formed during storage of insulin (rod shaped long structure with 1µm diameter). To prevent fibrillary accumulation, phospholipids and surfactants can be used at low concentration; the right pH can also help to avoid this undesirable phenomenon. To simulate basal and prandial insulin secretion, various pharmaceutical biotechnologies utilized to produce insulin analogs with different duration and onsets of action including amino acids sequence inversion, deletion and / or insertion.

Key wards: Insulin; Dimerization; Insulin analogs; Short acting; Intermediate acting; Long acting.

تقنيات الصياغة المستخدمة في تحضير وأنواع ومفعول نظائر الأنسولين: مراجعة موضوع

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الكلمات المفتاحية: الأنسولين; ديميريزيشن; نظائر الأنسولين; سريع الفعالية؛ وسيط التمثيل; طويل المفعول.

الخلاصة

الأنسولين هو هرمون ببتيد يتم إطلاقه كمؤيد للأنسولين من خلية البنكرياس بيتا (β) و يتكون من سلسلة A و B و C-peptide ، الأنسولين هو الذي يتكون بعد انقسام الببتيد C من الأنسولين المؤيد. استجابةً للوجبة ، يحدث إفراز الأنسولين المفرط لاستخدام الجلوكوز بينما يحافظ إفراز الأنسولين الأساسي على نسبة السكر في الدم. تعد القدرة الجوهرية للأنسولين على الارتباط السريع في الثنائيات وحالات النظام المتقدمة خاصية مهمة. في وجود الزنك ، تم استخدام القدرة على تشكيل السداسيات المنفصلة لإنشاء تركيبات الأنسولين ذات القيمة الطبية. يمكن الحصول على التغيير المطابق الذي يفضل الاستقرار الكيميائي من T6 إلى R6 عن طريق ربط السواغات الفينولية بمواقع فريدة على سداسي الأنسولين وهذا سيقلل من تراكم البوليمر عالي الوزن الجزيئي. من الخصائص المهمة الأخرى للأنسولين التي يمكن استغلالها أثناء التركيب العلاجي أن نقطة تساوي الأنسولين (PI) في حالة التحريف هي 5.3. يمكن تكوين تراكم ليفي أثناء تخزين الأنسولين (هيكل طويل على شكل قضيب بقطر 1 ميكرومتر). لمنع التراكم الليفي ، يمكن استخدام الفسفوليبيدات والمواد الخافضة للتوتر السطحي بتركيز منخفض ؛ يمكن أن يساعد PH الصحيح أيضًا في تجنب هذه الظاهرة غير المرغوب فيها. لمحاكاة إفراز الأنسولين الأساسي والانسولين بعد الوجبات ، يتم استخدام العديد من التقنيات الحيوية الصيدلانية لإنتاج نظائر الأنسولين لمدة مختلفة وبداية عمل بما في ذلك انعكاس تسلسل الأحماض الأمينية و / أو الحذف و / أو الإدخال.

Introduction

Insulin is a peptide hormone normally released from pancreatic β-cell in response to meal in addition to basal insulin secretion to maintain euglycemia after meal and during night, respectively [1]. It is released as proinsulin consist of A and B chain and C-peptide which is cleaved to form insulin [2], as seen in (figure 1).

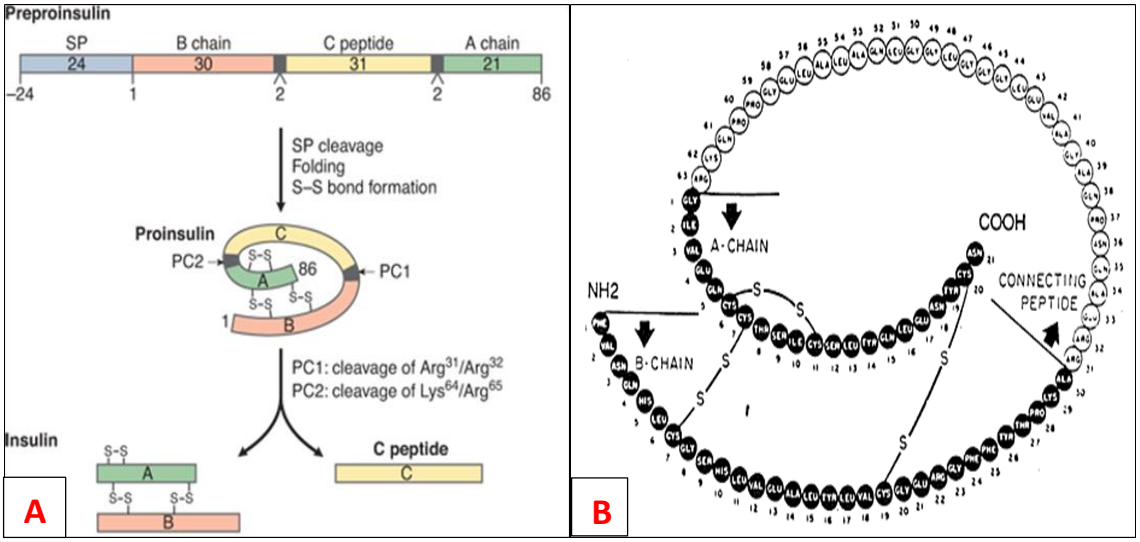

Figure 1: Structure of (A) Proinsulin and (B) **Natural insulin protein that consist of 51 amino acids, A-chain and a B-chain form a dimer through disulfide bonds [3].**

Insulin's intrinsic ability to quick association into dimers and advanced order states is an important property. The hydrophobic interactions formation at the B-C-end chains is a major driving force for dimerization [4]. In the existence of different metal ions, such as the divalent zinc (0.33 g-atom/monomer), insulin may form distinct hexameric

multiplexes, with each zinc ion synchronized by $HisB^{10}$ residue from three monomers [5]. In the presence of zinc, the ability to shape discrete hexamers has been used to create medicinally valuable insulin formulations. Antimicrobial phenolic excipients (e.g. m-cresol, phenol or methyl-paraben) are often used in commercial insulin preparations [6]. These excipients used as multipurpose agents acting as preservative against microbial growth, maintain sterility and insulin molecules stabilization to retard aggregation within hexameric structure [7]. A conformational change that favors chemical stability (from T6 to R6) can obtained by binding of phenolic excipients to unique sites on insulin hexamers and this will lessen the aggregation of high molecular weight polymer as seen in (figures 2 and 3) [8].

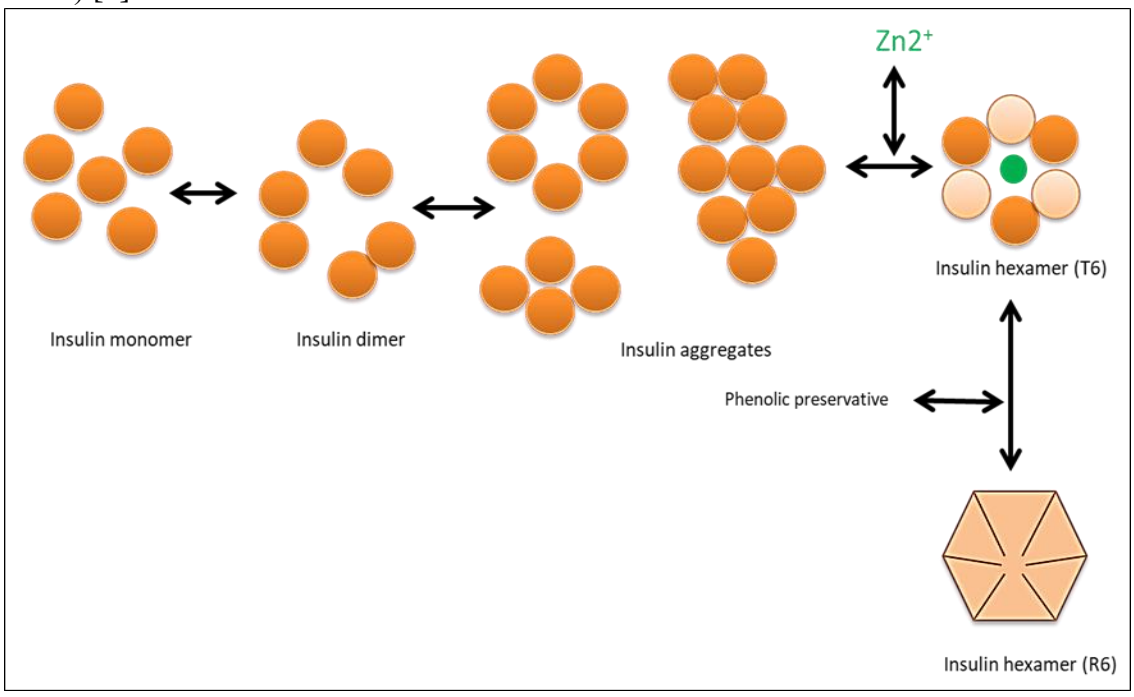

Figure 2: Dimerization of insulin in the existence of zinc and phenolic excipients [9].

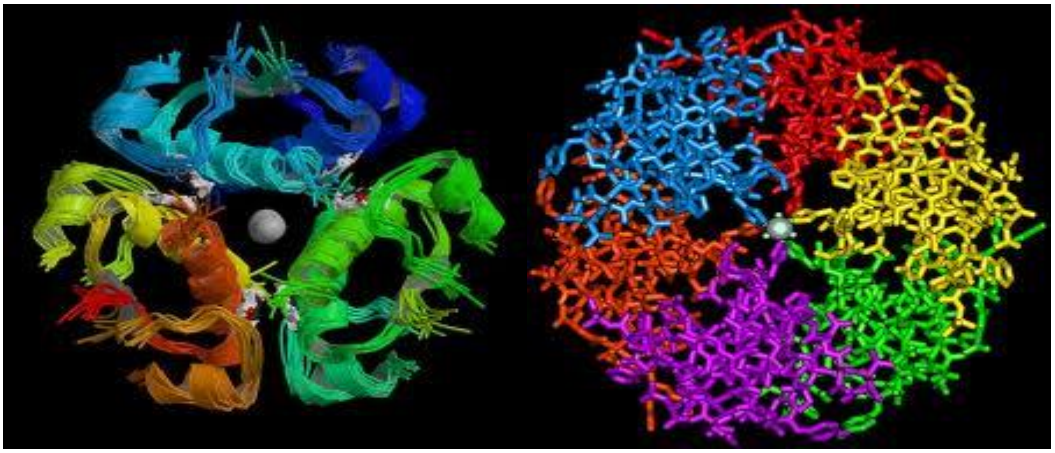

Figure 3: Hexameric association of insulin in the presence of phenol and zinc [10].

Another important property of insulin that can be exploit during therapeutic formulation is the insulin's isoelectric point (PI) in the denatured state is 5.3. Therefore, the insulin molecule is charged at neutral pH negatively and this can be used during formulation of insulin to avoid aggregation or precipitation [11]. PI defined as pH of a solution that makes the net primary load of a protein null. When PH of solution becomes above the PI the surface of the protein is mainly loaded with negative charge and the molecules with the same load show repulsive forces. At a pH solution below the PI, the protein surface is mostly loaded positively and protein repulsion occurs [12]. Negative and positive charges cancel at the PI, repulsive electrostatic forces are diminished, and attraction forces take precedence. Aggregation and precipitation would be caused by the attraction forces, as a result of the gain or loss of (H^+), the molecule is influenced by the pH of its surroundings and can become more positively or negatively charged [13]. At the pH that corresponds to their pI, such molecules have a minimum solubility in water or salt solutions and often precipitate out of solution. Rod-shaped long structure with small diameter less than 0.1 µm that is fibrillary precipitate can formed during insulin storage and can be solved or prevented by using phospholipid and surfactant at low concentration as they show anti-fibrillary effect [14]. Fibrillary aggregation can be formed during storage of insulin (rod shaped long structure with 1µm diameter) as shown if (figure 4) [15]. To prevent fibrillary accumulation, phospholipids and surfactants can be used at low concentration. The right pH can also help to avoid this undesirable phenomenon [16].

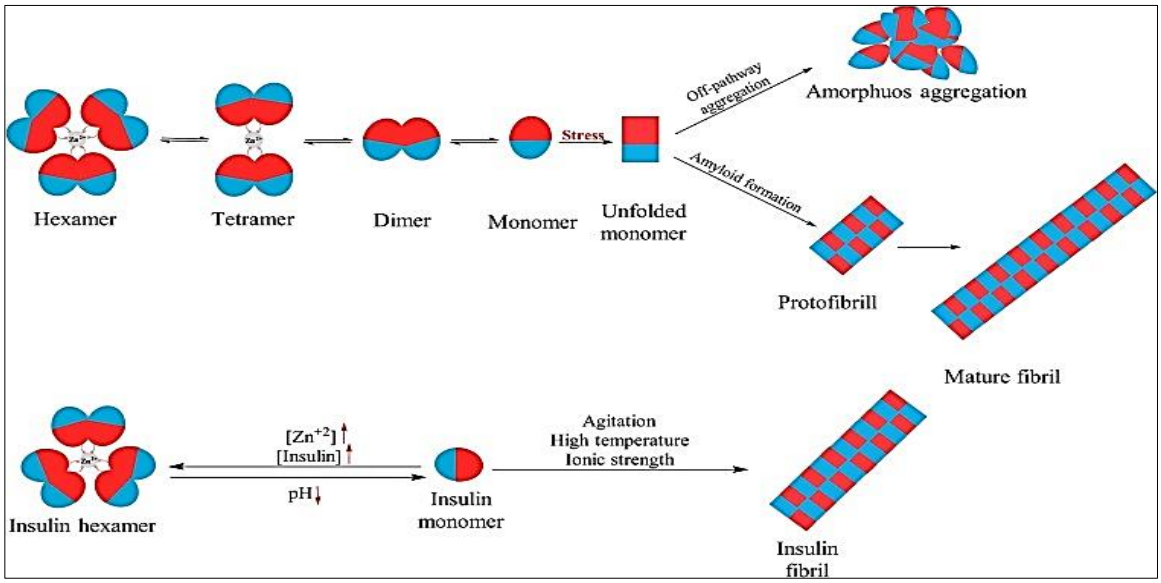

Figure 4: fibrillary precipitate formation during insulin storage [8]

Insulin Pharmacology and Formulations

Physiologic insulin exocytosis in normal non-diabetic individual categorized into prandial insulin secretion in response to a meal and basal insulin secretion that is constantly released between meals and during sleeping time [17]. Peak levels of serum insulin of 60-80 mU/mL are representative of the reaction of pancreas to a meal, while basal serum

insulin levels are usually 5-15 mU/mL [18]. Because of the wide range of insulin requirements, a great deal of work has gone into developing insulin formulations that satisfy the pharmacokinetic and pharmacodynamic necessities of each condition. To improve pharmacokinetic and pharmacodynamic properties, insulin analogs and insulin analog formulations have recently been fashioned [19].

Human Insulin

The FDA approved human insulin biosynthesis as the earliest recombinant DNA drug creation. Humulin (Lilly) was first made available in 1983. It is made with recombinant DNA technology and a non-pathogenic laboratory strain of Escherichia coli [20]. The bacteria are inoculated with plasmid recombined to DNA carrying gene coding for humanoid insulin, which is then fermented to generate the A and B chains of humanoid insulin. Individually, before being joined by disulfide bridges to form human insulin, the A and B chains are released and purified. Chemically, physically, and immunologically, the insulin produced is identical to insulin extracted from the pancreatic cells of human. The resultant recombinant product of insulin is devoid of E. coli as well as pancreatic peptides contaminants, which can be found as impurities in insulin formulations produced from animal pancreatic sources [21, 22]. Proinsulin and glucagon, proinsulin intermediates, pancreatic polypeptide, somatostatin and vasoactive intestinal peptide are among the above impurities. Human insulin was first sold in two forms: neutral standard human insulin (Humulin R, by Lilly) and NPH human insulin (Humulin N, from Lilly). Zinc-insulin crystals in solution make up neutral regular human insulin. It starts quickly and has a short time to work (6 to 8 hours) [23]. NPH human insulin is a slow start and a longer action time (16 to 20 hours) turbid medium-acting insulin compared to normal insulin. In a cold environment, a refrigerator is preferred at 2 – 8 ºC, human insulin should be stored to retain original quality till expiration date. However, insulin preparations can stored at 25 ºC or less upon using for limited duration ranged from ten days upto two months. All types of insulin should circumvent freezing [24, 25].

Short-Acting Insulin Formulations

Regular Insulin

Insulin in a form of sterile aqueous solution is known as regular insulin. Amorphous insulin was the first insulin produced for clinical use. A purer zinc-insulin crystalline that provided a clear aqueous solution eventually replaced this form. Insulin injection (regular insulin) was initially made at a pH of 2.8 to 3.5. When the pH was raised above the acid range, particles formed in the vial, necessitating this step [26]. However, advances in processing to manufacture more purified insulin have made it possible to inject insulin with a neutral pH. The stability of acidic insulin formulation is less than the neutral product.

The neutral, standard formulations have a median length of 5 to 8 hours and peak insulin production between 2 and 4 hours. Time- action can vary in response according to variables such as dosage, injection location, temperature, and physical activity of the patient, just as they can be with other formulations [27]. Despite the fact that the insulin in these formulations is soluble, there is still a pause in activity. Dissociation of hexamer to dimer

and monomer is required for absorption, this may appear some delay in the onset of insulin action [28]. The preservative and insulin must diffuse away from the injection site, essentially diluting the protein and changing the balance from hexamers to dimers and monomers as illustrated in (figure 5). Recent study studies investigating the molecular weight and cumulative doses of various compounds recovered from subcutaneous injection in the popliteal lymph show that transport through lymph system can excuse for about twenty percent of insulin intake from interstitium, the rest of the insulin balance is mainly absorbed by capillary diffusion [29].

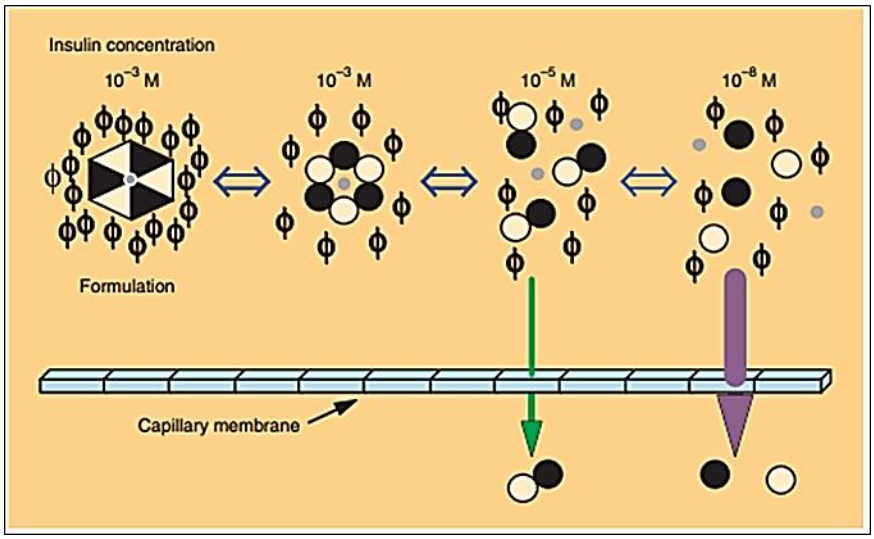

Figure 5: insulin dissociation, dilution and diffusion via capillary membrane [30].

Analogues of insulin monomer were created to provide patients with a more natural body reaction to meal glucose load while still being convenient. The aim of developing monomeric insulin analogs for the treatment of insulin-dependent diabetes mellitus has been to change insulin's self-association properties in favor of the monomeric species, reducing time-action latency [31]. $LysB^{28}$ $ProB^{29}$-human insulin is one such monomeric analog that has been developed and has a faster time-response profile with an ultimate activity of about 1 hour [32]. Inversion of the sequence at positions B^{28} and B^{29} results in an analog with less self-association activity than human insulin; but insulin lispro is stabilizing in a hexameric complex depending on preservatives, which ensures the required chemical and physical stability required of insulin preparations.

Insulin Lispro

Insulin lispro solution consists of a clear aqueous fluid of zinc-insulin lispro crystals. The amino acids are created when the insulin-B chain is reversed at positions 28 and 29. Insulin lispro is one of the insulin analog in which the reversed proline at location B^{28} with B^{29} lysine [33]. It is dispensed as subacute as a bolus with the use of selected external controlled infusion devices, using conventional syringe or continuous subcutaneous infusion. Although this analog is hexamerically complex, insulin lispro retains its quick action time [34]. In 1995, Ciszak et al assumed that the decreased dimerising characteristics

of the correspondent, together with preservative reliance, yields a hexameric complex which easily dislocates into monomers after the antimicrobial phenol preservative is disseminated quickly into the subcutaneous tissue at the injecting place (figure 6). Therefore, dissociation of hexamer to dimers and monomers is essential for absorption, the significant dilution (10^5) of human insulin zinc-hexamer is not crucial [35].

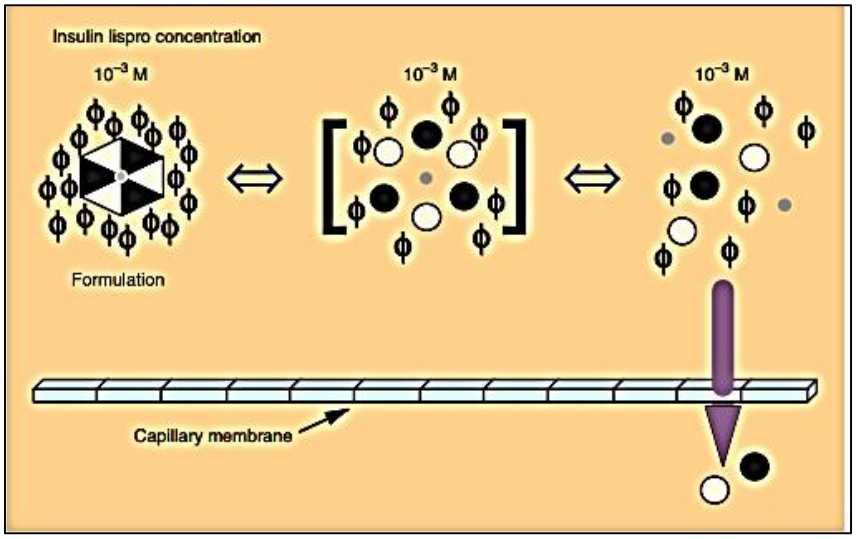

Figure 6: insulin lispro diffusion through capillary membrane [30].

Insulin Aspart

Insulin aspart is a recombinant, ultra-short insulin that uses the production organism of *Saccharomyces cerevisiae*. It is consistent with normal human insulin with the exception of one single aspartic acid replaced the amino acid proline in B^{28}. It is usually co-administered to satisfy basal insulin needs for diabetic patients with certain long-acting insulins (e.g. NPH) [36].

Insulin Glulisine

Glulisine insulin is a fast acting, recombined insulin-based analog varies from insulin human by replacing amino acids of the beta-chain at B3 and B29 (lysine instead of asparagine and glutamic acid instead of lysine) respectively. This insulin is produced by a nonpathogenic strain of E. coli using recombinant DNA technology [19].

It is compatible with regular human insulin after intravenous administration. Glulisine is given subcutaneously and shows a faster onset of action and a shorter time than normal insulin. The solution with a pH approximately of 7.3 is sterile, clear, aqueous and colourless. It is unlike the other rapid-acting analogues, in which it is formulated in the presence of a stabilizer like polysorbate 20 instead of zinc-insulin formulation [35, 37].

The surfactant in the formulation is thought to reduce higher order interaction and speed up the adsorption of monomeric species. Manufacturers have developed soluble formulations for use in external or implanted infusion pumps, in addition to the aforementioned rapid-acting formulations [38]. These formulations are very similar to regular insulin in most ways (hexameric association state, preservative, and zinc).

However, buffers and/or surfactants can be added to these formulations to prevent physical accumulation of insulin, which may cause infusion sets to clog [39]. External infusion pumps are licensed for use with all three commercially available rapid-acting insulin analogs.

Insulin Technosphere

Technosphere insulin is a recombinant human insulin that presented as dry-powder formulation for inhalation and absorption by pulmonary tissue [40]. It is a fast-acting powder insulin that is inhaled at mealtimes to help type 1 and type 2 diabetics for better regulate their blood sugar levels. In diabetic ketoacidosis, the patient does not use technosphere as a replacement for long-acting insulin. In type 1 diabetes, it must be combined with long-acting insulin. Technosphere insulin is a tiny whistle-like system that comes in a single-use color-coded cartridge that provides 4, 8, or 12 units of insulin shortly before a meal as seen in figure 7. Technosphere should be inhaled as a single inhalation per cartridge using an inhaler at the start of a meal [41, 42].

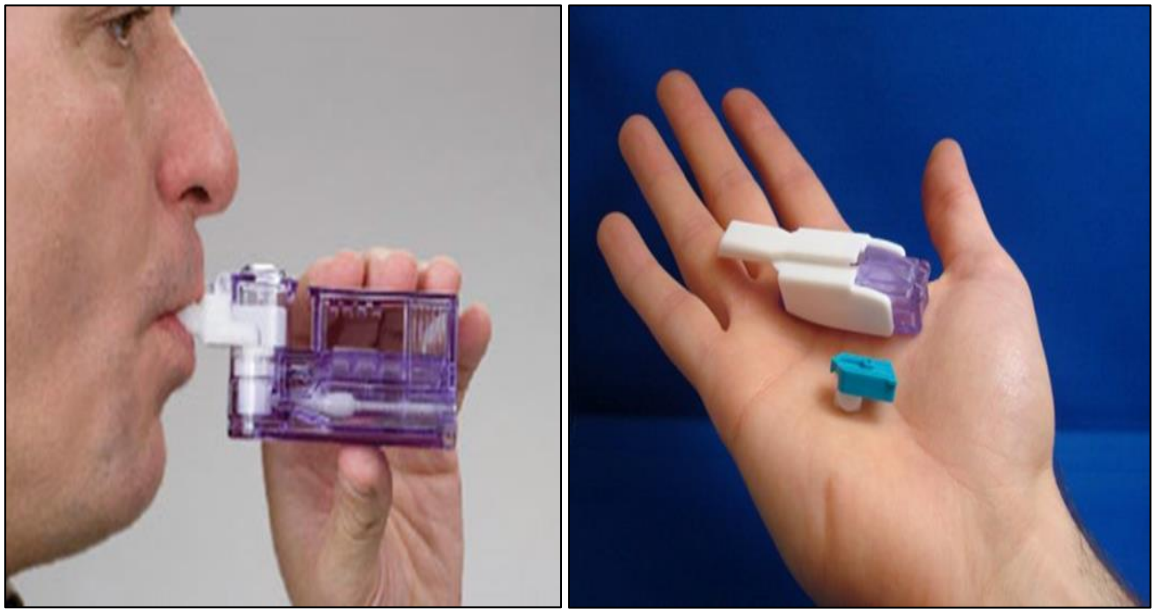

Figure 7: technosphere insulin and the whistle cartilage [43].

Intermediate-Acting Insulin Formulations

Insulin (NPH) and Lente

Neutral Protamine Hagedorn (NPH) and Lente are the two widely used intermediate acting preparations of insulin. Both formulations require the dissolution of a precipitated and/or crystalline form of insulin to achieve prolonged time-action [44]. Mostly, the limiting step in absorption rate of intermediate- and long-acting insulin is dissolution. As a result, the formulation duration extended by suspending the cleaved parts of hexamer, into dimers and monomers. NPH is a neutral crystalline suspension made by co-crystallization of insulin and protamine; its name belong to discoverer H.C. Hagedorn [45].

Insulin suspension Isophane

Suspension of isophane insulin to a watery vehicle with dibasic sodium phosphate buffered to pH 7.1 to 7.4 is sterile suspension. It consists of zinc-insulin crystals, which were modified with protamine into a compound of insulin, Zinc and protamine crystals during the solid stage of the suspension [46]. The term isophane comes from the Greek terms "*iso*" (equal) and "*phane*" (appearance), and it refers to the protamine and insulin balance being equal.

Long-Acting Insulin Formulations

To retain basal glycemic function, the human pancreas secretes about one unit of insulin (0.035 mg) per hour [47]. The pharmacokinetic profile of long-acting insulin formulation must be very different from that of "meal-time" insulin formulation. Ultralente, which was produced in the 1950s, and two insulin analogs, Lantus (insulin glargine) and Levemir (insulin detemir), are the three long-acting insulin preparations currently available in the market [48].

Insulin Glargine

Insulin glargine marketed under trade name Lantus® that given once daily subcutaneously at night for the management of diabetes mellitus type 1 in adults and children with a long-acting (up to 24 hours) basal insulin preparation. Adults with type 2 diabetes that use long-acting insulin may also use it [49]. It is formed when glycine replace the amino acids at location 21 of human insulin and adding two arginines to the B chain's C terminus. The extra arginine residues cause the pI to move from 5.4 to 6.7, resulting in an insulin correspondent that is soluble at acidic pH values but less soluble at subcutaneous tissue when the PH is neutral [50]. Insulin glargine is a human insulin analog that is produced using recombinant technology. It is fully soluble at pH 4.0, where it is formulated. However, it is neutralized until injected into SC tissue, resulting in the creation of microspheres (a slowly dissolving precipitate). These consequences in a moderately continuous absorption rate over the course of 10.8 to 24 hours, with no discernible peak [51, 52]. It allows once-daily dosing for attaining patient's basal insulin. Since native asparagine is vulnerable to acid-mediated degradation and shrunk potency, the addition of glycine at location A21 results in a product with adequate chemical stability under acidic formulation conditions. As a result, improvements to insulin's molecular sequence have been prepared to increase chemical stability and modify absorption from subcutaneous tissue, resulting equipotent analog with human insulin [53].

Insulin Detemir

Detemir insulin, trade name Levemir®, is a given once or twice daily subcutaneously as intermediate- to long-acting insulin. It is generated from recombinant DNA utilizing *S. cerevisiae* (baker's yeast) and comes as a simple neutral (PH 7.4) solution [54]. With the exception of a removal of the amino acid threonine in position B30 and a C14 fatty acid chain added to the amino acid position B29, it is structurally similar to human insulin. Insulin detemir's long-acting property is maintained by slow systemic absorption. Insulin

detemir works by acylating insulin with a fatty acid moiety to produce a long-lasting pharmacological effect. In a retained solution, the analog procedures a zinc hexamer at neutral PH [55].

Insulin Degludec

It is an insulin analogue with a 42-hour half-life since B-30 threonine has been removed and B-29 has been conjugated to hexadecanedioic acid. The multi-hexamers complexes produced at the site of injection and the slow release of monomers account for the long time of operation. A subcutaneous injection of insulin mixture consisting of insulin degludec and insulin aspart at (70/30) used for intermediate action [56].

Conclusion

Biopharmaceutical techniques used to manipulate amino acid sequence by inversion, deletion and/or insertion for the production of insulin analogs with controlled onsets and durations of action. Consequently, these analogs can be used for the management of diabetes mellitus type1 and in some occasion of type 2 to simulate basal and prandial insulin secretion. Pharmaceutical biotechnologies exploit the natural insulin property of dimerization and thereby producing insulin with more stable conformation during storage. Additionally, it can be easily diluted with optimized penetration through percutaneous layers of skin.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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