Review of Insulin and its Analogues in Diabetes Mellitus

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ABSTRACT

Diabetes is a metabolic disorder where in human body does not produce or properly uses insulin, a hormone that is required to convert sugar, starches and other food into energy. Diabetes finally leads to more complications and to prevent these complications insulin and its analogues are used. After more than half a century of treating diabetics with animal insulin's, recombinant DNA technologies and advanced protein chemistry made human insulin preparations available in the early 1980s. As the next step, over the last decade, insulin analogues were constructed by changing the structure of the native protein with the goal of improving the therapeutic properties of it, because the pharmacokinetic characteristics of rapid, intermediate and long-acting preparations of human insulin make it almost impossible to achieve sustained normoglycemia. The first clinically available insulin analogue, lispro, confirmed the hopes by showing that improved glycaemic control can be achieved without an increase in hypoglycaemic events. Two new insulin analogues, insulin glargine and insulin aspart, have recently been approved for clinical use in the United States and several other analogues are being intensively tested.

DIABETES MELLITUS

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both [1-4]. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism [1-4]. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy [5,6], neuropathy [7,8], nephropathy [9,10], Cardiovascular complications [11,12] and ulceration [13,14]. Thus diabetes covers a wide range of heterogeneous diseases. Diabetes is the most common endocrine disorder and by the year 2010, it was estimated that more than 200 million people worldwide had DM and 300 million will subsequently have the disease by 2025 [15-17]. The diagnostic criteria and the classification of diabetes was first put forward by the World Health Organization (WHO) in 1965[18] then by the National Diabetes Data Group (NDDG) in 1979 [19] and this was followed by simplified recommendations by the WHO in 1980 [20]. These WHO recommendations were modified slightly in 1985 [21]. The latest recommendations have been published by the American Diabetes Association (ADA) in 1997 and by the WHO in 1999. Both groups agree on the recommendations and criteria [2, 22].

According to the ADA recommendation changes in 1997, the fasting glucose concentration should be used in routine screening for diabetes as well as epidemiological studies; the threshold for fasting glucose was changed from 7.8 mmol/L (140 mg/dl) to 7.0 mmol/L (126 mg/dl); however the 2 hrs glucose criterion remains as 11.1 mmol/L (200 mg/dL). For the diagnosis of diabetes, at least one of the below criteria must apply.

- Symptoms of diabetes (polyuria, polydipsia, unexplained weight loss, etc) as well as casual plasma glucose concentration = 11.1 mmol/L (200 mg/dL).

- Fasting plasma glucose = 7.0 mmol/L (126 mg/dL), with no caloric intake for at least 8 hrs.
- 2 hrs plasma glucose = 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test (OGTT), with the glucose load containing 75 g anhydrous glucose in water.

The WHO diagnosis and classification of diabetes mellitus (1999) are identical to those of ADA, a fasting glucose = 7.0 mmol/L (126 mg/dl) and /or a 2 hrs glucose = 11.1 mmol/L (200 mg/dL). The report states that diagnosis should not be based on a single glucose determination but requires confirmatory symptoms or blood/plasma determination. Ideally therefore, both the 2 hrs and fasting value should be used. These recommendations contrast with those of ADA Expert Committee which gives primacy to the fasting plasma glucose. The WHO classification includes both clinical stages (normoglycaemia, impaired glucose tolerance/impaired fasting glucose (IGT/IFG), diabetes and aetiological types of diabetes mellitus, identical to the ADA except that WHO group includes classification formerly known as gestational impaired glucose tolerance (GIGT) and GDM: fasting glucose = 7.0 mmol/L (126 mg/dL) and/or 2 hrs glucose = 7.8 mmol/ L (140 mg/dL) after a 75-g OGTT.

Diabetes mellitus may be categorized into several types but the two major types are type I and type II [21, 23]. On the basis of aetiology, the term type I and type II were widely used to describe IDDM and NIDDM, respectively. The term juvenile -onset diabetes has sometimes been used for IDDM and maturity-onset for NIDDM.

Type I (Ia, Ib) ß-cell destruction with little or no endogenous insulin secretory capacity Autoimmune Idiopathic Type II Ranges from relative insulin deficiency to disorders of insulin secretion and insulin resistance.

On the basis of etiology, type I is present in patients who have little or no endogenous insulin secretory capacity and who therefore require insulin therapy for survival. The two main forms of clinical type I diabetes are type Ia (about 90% of type I cases in Europe) which is thought

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Figure 1: The Discovery of Insulin and its analogues.
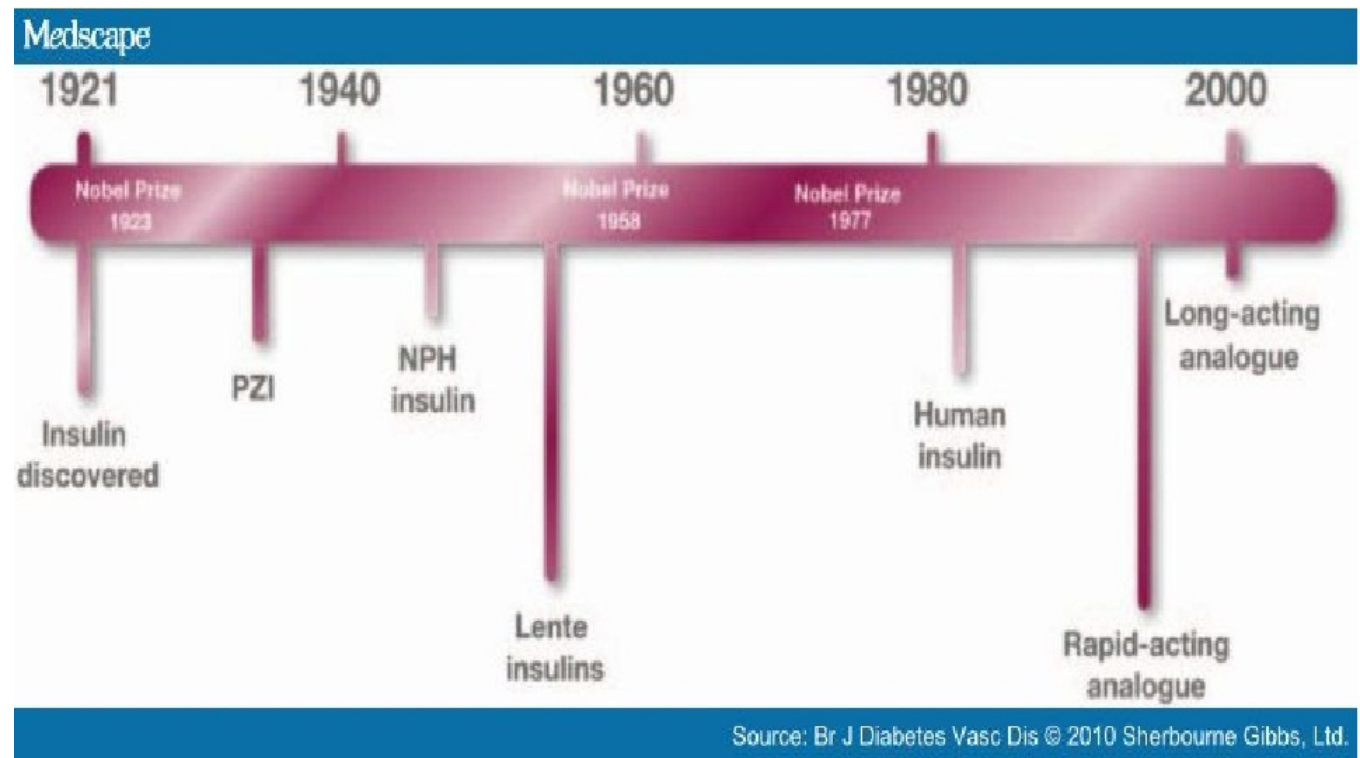
Figure 2: Structure of insulin.
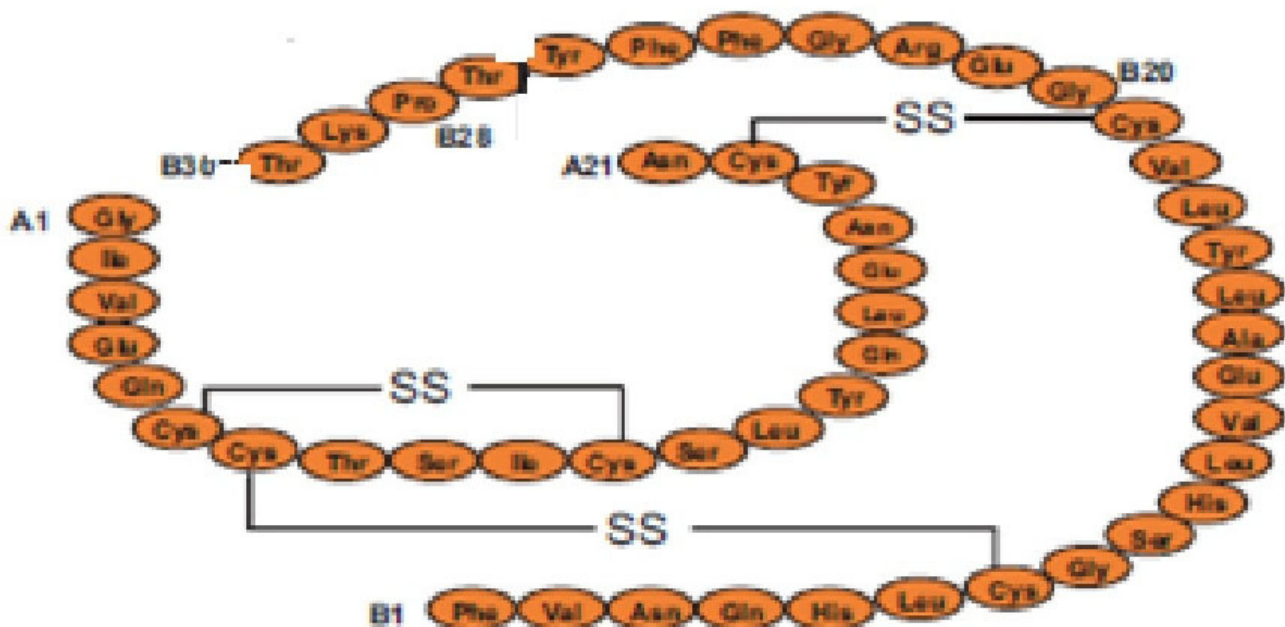
therapy. When the hormone is absorbed and the concentration falls to physiological levels (nanomolar), the hormone dissociates into monomers and the monomer is most likely the biologically active form of insulin. Monomeric insulin is now available for therapy.

Substantial information about the structure–activity relationship of insulin has been obtained by study of insulin's purified from a wide variety of species and by modification of the molecule. A dozen invariant residues in the A and B chains form a surface that interacts with the insulin receptor. These residues—Gly^{A1}, Glu^{A4}, Gln^{A5}, Tyr^{A19}, Asn^{A21}, Val^{B12}, Tyr^{B16}, Gly^{B23}, Phe^{B24}, Phe^{B25}, and Tyr^{B26}—overlap with domains that also are involved in insulin dimerization. The Leu^{A13} and Leu^{B17} residues

may form part of a second binding surface. Insulin binds to surfaces located at the N- and C-terminal regions of the subunit of the receptor, including a cysteine-rich region in the receptor chain. In most cases, the affinity of insulin for the insulin receptor correlates closely with its potency for eliciting effects on glucose metabolism. However human, bovine and porcine insulin's are equipotent [56, 57].

Insulin Analogues

In no diabetic individuals, ingestion of food results in a relatively rapid rise of serum insulin concentration to a maximum after 30–45 min followed by a decline to basal levels after 2–3 hrs. The pharmacokinetic

characteristics of the currently available rapid, intermediate and long-acting preparations of human insulin make it almost impossible to achieve sustained normoglycemia. The onset of action of SC-injected regular human insulin is too slow and the duration of its action too long to mimic the insulin secretion pattern of a healthy individual during ingestion of a carbohydrate- containing meal [58]. As a result, early postprandial hyperglycaemia followed by an increased risk for hypoglycaemia before the next meal are present. Similarly the available intermediate/long-acting human insulin preparations are unable to provide a stable, continuous baseline insulin level. Instead they cause peak serum insulin levels at 3–4 hrs after SC injection and show considerable inter- and intrasubject variations in their bioavailability. The Diabetes Control and Complications Trial confirmed the link between glycaemic control and the complications of diabetes [59]. Therefore, to achieve improved glucose control, the need for new insulin preparations with a faster onset and shorter duration of action and for long-acting preparations with a more flat time-action profile and less variable bioavailability became apparent in the late 1980s and early 1990s [60]. However, until recently, improvements in insulin formulations were seriously limited; advances were only achieved in insulin purity, species and characteristics of the retarding agent. The availability of molecular genetic techniques opened new windows for creating insulin analogues by changing the structure of the native protein, improving its therapeutic properties. In addition to its glucose lowering effect, insulin is the most potent physiological anabolic agent known to date [61]. It promotes the synthesis and storage of lipids, proteins and carbohydrates and prevents their degradation and release back to the circulation. Despite years of intensive investigation, we are still left with considerable uncertainty regarding the precise intracellular events that mediate the action of this hormone. One confounding factor has been the variety of actions of insulin, which depend on the cell type, time of exposure, and the presence or absence of other hormones [62]. Another is the fact that insulin can act as a growth factor for cultured cells and shares many of the mitogenic signalling pathways elicited by other growth factors. However, the metabolic effects of insu-

lin are unique and cannot be reproduced by other cellular stimuli [60, 63]. Taken together, these findings indicate that signalling mechanisms that respond only to insulin exist, and they allow for the specialized effects of insulin on metabolism. Designing and studying insulin analogues has helped and without any doubt will help, our understanding of the complex processes insulin is associated with and creating analogues selective to one or another of insulin's actions might well be of clinical significance.

Insulin lispro (Eli Lilly & Co., Indianapolis, IN)
Scientific Information:

- Chemical Name: Lys(B28),Pro(B29) -human insulin
- Molecular weight: 5808 Dalton's
- Molecular Formula: $C_{257}H_{383}N_{65}S_6O_{77}$

The B26 –30 region of the insulin molecule is not critical in binding to the insulin receptor. However, it is clearly important in mediating the formation of insulin dimers [66]. Therefore structural modifications of the molecule at these positions would be expected to generate insulin analogs with minimal tendency for self-association but unaltered affinity to the insulin receptor compared with regular human insulin [65]. The first genetically engineered rapid-acting insulin analogue to become available for the clinician was insulin lispro, which was approved for clinical use in Europe in April of 1996 and in the United States in June of 1996. In insulin lispro, the normal sequence of proline at position 28 of the B chain and lysine at position 29 is reversed (**Figure 3**). This reversal causes a decreased tendency for self-association and as a result faster absorption, higher peak serum levels and shorter duration of action can be observed with insulin lispro compared with regular insulin. Importantly, as discussed above, the amino acid sequence changes in lispro do not affect its receptor-binding domain. Therefore the affinity to the insulin receptor of insulin lispro is similar to that of regular insulin. Although lispro affinity for the IGF-I receptor is slightly higher, it is not enough to cause a difference in its cell growth-stimulating activity compared with regular insulin [68, 69].

Figure 3: Modifications of the Insulin Sequence in Insulin Lispro.
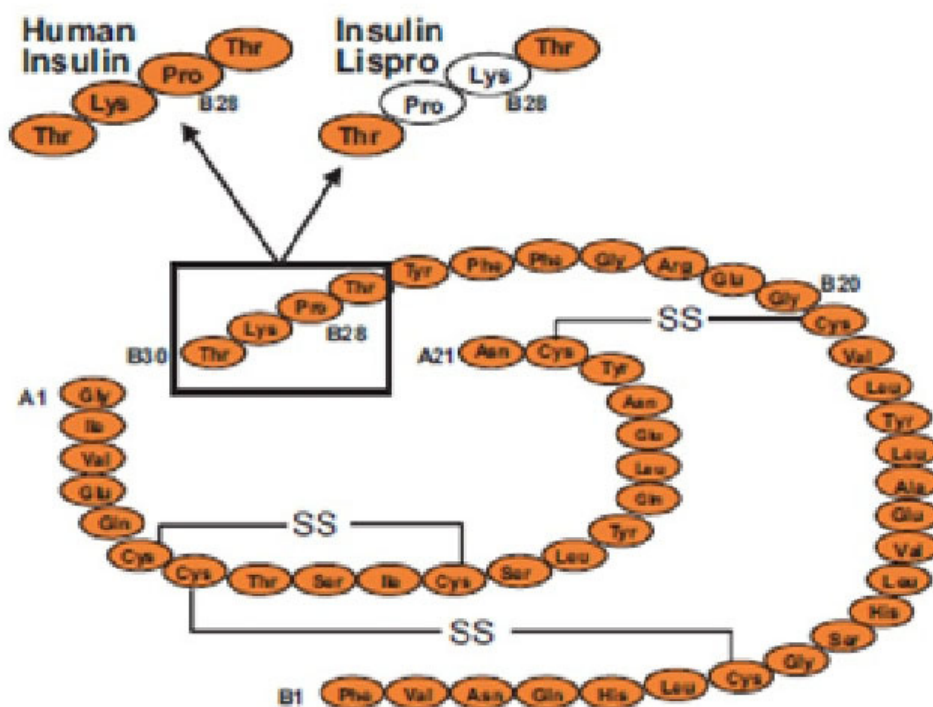
In terms of activity on lipogenesis, insulin lispro was found to be essentially the same as human insulin [64]. Pharmacokinetic studies indicate that insulin lispro acts within 15 min, peaks in approximately 1 hrs and disappears within 2–4 hrs after SC injection [67, 70]. In clinical studies, as expected from a short-acting analogue, insulin lispro achieved significant improvements in postprandial glucose levels with a lower rate of hypoglycaemic events compared with regular insulin [71-73]. This can be observed even if insulin lispro is administered immediately before meals and regular insulin is injected 30–45 min before meals. Unfortunately, in most cases these beneficial effects were not accompanied by improvements in glycosylated haemoglobin values [71, 72]. In addition to the decrease in hypoglycaemic events, the most likely explanation for this is the inability of the currently used long-acting insulin's to provide true basal coverage. Therefore, increased pre-prandial plasma glucose concentrations are present in patients on insulin lispro. Supporting this theory, a clinically and statistically significant decrease of hemoglobinA1c levels was seen when insulin lispro was used with two or more daily injections, instead of one, of neutral protamine Hagendorn (NPH) insulin [74, 75]. Therefore, for the intensive therapy of diabetes by multiple daily injections, the addition of a few units of NPH to lispro at each meal, combined with bedtime NPH, can be recommended [75-77]. This regimen may even improve unawareness of and impaired counter regulation to hypoglycaemia [77]. Insulin lispro has also been tested for use in pregnancy and gestational diabetes [78, 79].

Based on the limited available data on its long-term effectiveness, it appears that insulin lispro remains effective in treating diabetic patients up to 5.4 years of treatment [80]. No differences have been reported between insulin lispro and regular insulin in the likelihood of developing allergic reactions, adverse events or abnormal laboratory values [81]. The immunogenicity of insulin lispro is similar to that of regular insulin [82]. Antibodies specific against insulin lispro hardly ever develop and do not affect dose requirements [80, 83]. Interestingly, there have been reports of patients in whom severe resistance to human insulin due to antibody formation was successfully overcome by switching them to insulin lispro [84, 85].

Insulin Glargine [HOE 901, LANTUS (Aventis Pharmaceuticals, Parsippany, NJ)]

Insulin glargine is a recombinant human insulin analogue produced by DNA technology using non-pathogenic strains of *Escherichia coli, Pichia pastoris*.

HOE 901 (insulin glargine, LANTUS) is a new long-acting biosynthetic human insulin analogue developed by Aventis Pharmaceuticals, which was approved for use in patients with type I and type II diabetes mellitus by the United States Food and Drug Administration in April of 2000 and by the European Agency for the Evaluation of Medicinal Products in June of 2000 [86,87].

Scientific Information:

- Chemical Name: 21^A-Gly-30^Ba-L-Arg-30^Bb-L-Arg-human insulin
- Molecular weight: 6063 Dalton's
- Molecular Formula: $C_{267}H_{404}N_{72}S_6O_{78}$

Two modifications of human insulin result in a stable molecule which is soluble in slightly acidic conditions (pH 4.0) and precipitates in the neutral pH of subcutaneous tissue. Because of these properties, absorption of insulin glargine is delayed and the analogue provides a fairly constant, basal insulin supply without peaks in plasma insulin levels for approximately 24 hrs, similar to that achieved by a continuous subcutaneous insulin infusion [86, 87].

The structure was designed by substituting an asparagine residue with a glycine at position 21 of the A-chain and elongating the B-chain at the C-terminus by addition of 2 arginine residues (**Figure 4**). Modification of B-chain caused the pH to shift from 5.4 to 6.7 and makes it less soluble at physiological pH and more soluble at acidic pH. The glycine substitution of A chain of insulin glargine stabilizes the hexamer structure and therefore, contributing to delayed delivery from subcutaneous depot and maintaining its stability in acidic solution. Insulin glargine is not to be mixed with other insulin, as it becomes cloudy and results in alteration of pharmacokinetic and pharmacodynamics profile. It precipitates at physiological pH and absorbs slowly from injection site.

Figure 4: Modifications of the Insulin Sequence in Insulin Glargine.
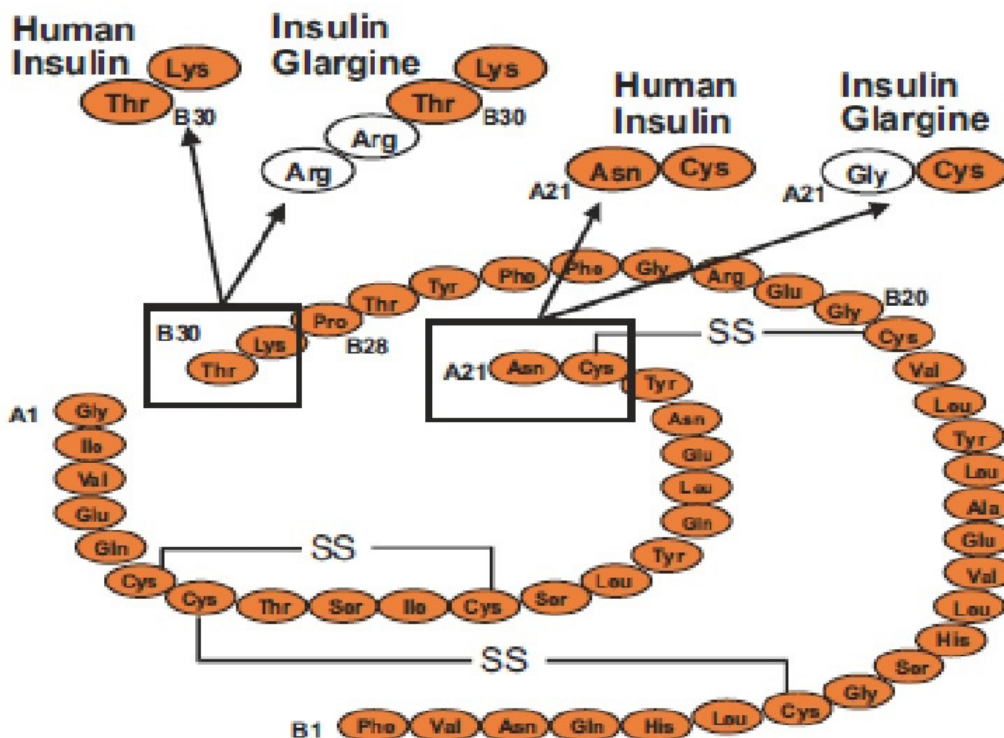
After SC injection insulin glargine precipitates in the SC tissues, which delays its absorption and prolongs its duration of action [88]. The substitution at position A21 largely increased the bioavailability of this analogue, so unlike Novo Sol Basal, it is suitable for clinical use [89]. With respect to insulin receptor binding, receptor auto phosphorylation, phosphorylation of signalling elements, and promotion of mitogenesis in muscle cells, insulin glargine behaves like regular human insulin [90]. Moreover, the growth-promoting activity of HOE 901 in muscle cells and the maximal metabolic activity of this analogue are not different from those of native human insulin; whereas its lipogenic activity is slightly lower [91]. However, insulin glargine therapeutic properties and potentials are remarkable and different from human insulin. HOE 901 was shown to exert a glucose-lowering effect for 24 hrs after a single daily injection without a pronounced plasma peak and induced a smoother metabolic effect than NPH insulin [88, 92]. Thus HOE 901 is expected to better substitute basal insulin requirements. Moreover, although it is well known from clinical practice that the effect of NPH insulin can vary with the site of injection, it has been found that changes in the injection site do not alter the time-action profile of HOE 901[93, 94]. In one of the first small, short-term clinical studies investigating this analogue in 1996, once-daily injections of HOE 901 resulted in similar glycaemic control as compared with four daily injections of the same total units of NPH in type I diabetics [95]. The characteristics of HOE 901 have been investigated in both type I and type II diabetic patients. In phase II trials conducted in Europe and the United States with type I diabetics, once-daily injections of HOE 901 along with premeal regular insulin achieved significantly lower fasting plasma glucose levels [96] and hemoglobinA1c values compared with patients on NPH and regular insulin [97]. Remarkably, the better glucose control was associated with similar or even lower incidences of hypoglycaemia. Studies of type 2 diabetic subjects showed similar fasting plasma glucose values with one injection of HOE 901 compared with those found with one or two injections of NPH insulin. Again, the incidence of hypoglycaemia was similar or lower among patients on HOE 901[98-100]. More recently, the findings of less frequent hypoglycaemic episodes and lower fasting plasma glucose levels

compared with NPH were confirmed in large, multicentre clinical trials with type I and type II diabetics in Europe and the United States [101-104]. Considering that less hypoglycaemia was consistently observed, these data suggest that the target fasting plasma glucose level can be lower for insulin glargine than for NPH [104]. The technical difficulties with blinding the studies comparing NPH and HOE 901 should be noted, as the two preparations can be easily identified because HOE 901 is a clear solution as opposed to the cloudy solution of NPH. It might make designing blinded research studies more difficult, but in daily clinical life it could actually be an advantage that insulin glargine is a clear solution. It has been shown that patients do not sufficiently shake suspensions like NPH insulin before administration [105]. Because it is not necessary to shake HOE 901 before usage it may have a lower intraindividual variability of its metabolic effect. In recent clinical trials patients treated with insulin glargine had less variability of their fasting plasma glucose values than those receiving.

Insulin Aspart [Novo Log (Novo Nordisk, Princeton, NJ)

In insulin aspart, substitution of proline (**Figure 5**) with the charged aspartic acid is carried out to reduce self-association of the molecule [106]. Like lispro it is a short acting analogue. Novo Nordisk created "aspart" and marketed it as Novo Log/Novo Rapid (UK-CAN) as a rapid acting insulin analogue. It was created through recombinant DNA technology so that the amino acid B28, which is normally proline, is substituted with an aspartic acid residue. The sequence was inserted into the yeast genome and the yeast expressed the insulin analogue, which was then harvested from a bioreactor. This analogue also prevents the formation of hexamer to create faster acting insulin. This analogue was approved for clinical use in the United States in June of 2000. Preclinical studies of insulin aspart have demonstrated that receptor interaction kinetics with the insulin receptor and with the IGF-I receptor are essentially equivalent to those seen with human insulin [107] and an equivalent metabolic effect of insulin aspart and human insulin has been shown with iv administration [108]. The potency on lipogenesis of insulin aspart is similar to that on human insulin whereas its affinity

Figure 5: Modifications of the Insulin Sequence in Insulin Aspart.
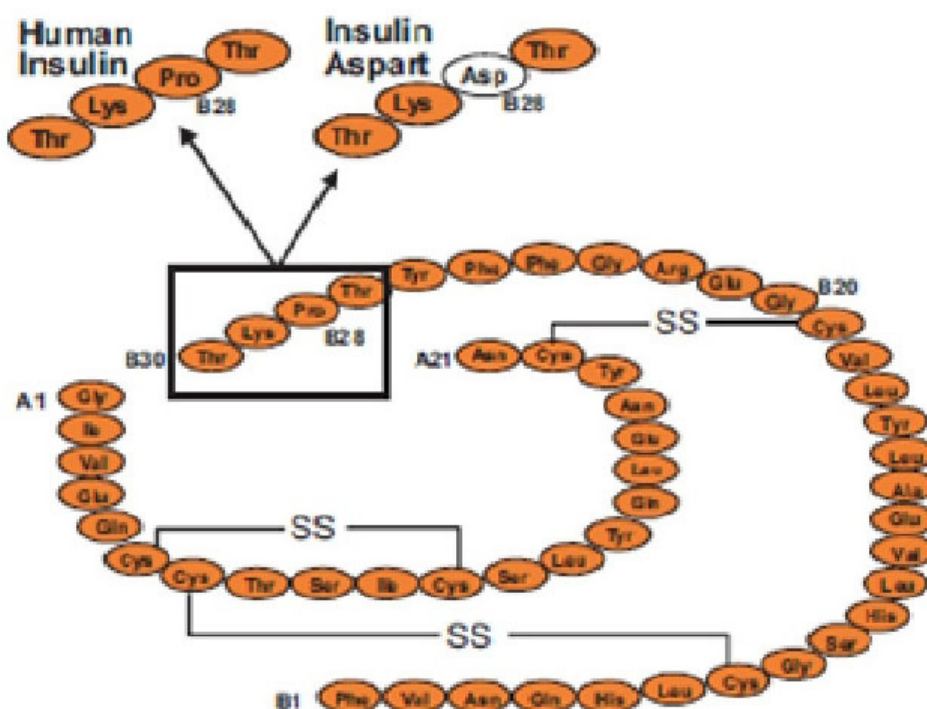
Figure 6: Modifications of the Insulin Sequence in Insulin Detemir.
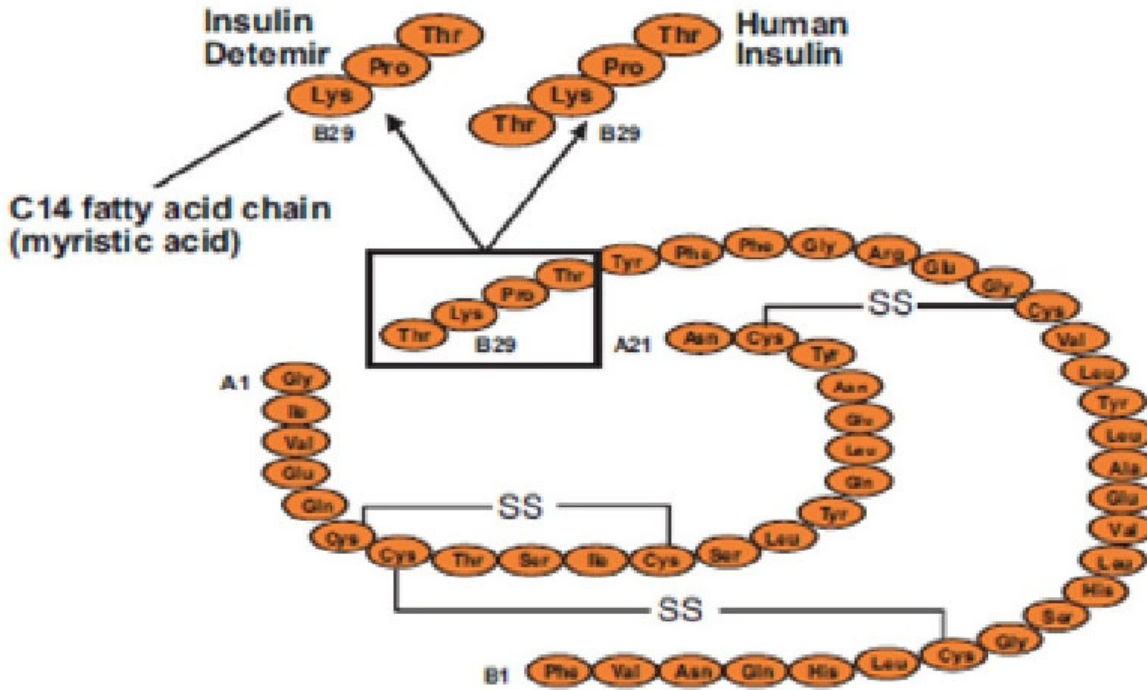
to the IGF-I receptor is slightly lower and thus it does not result in greater mitogenic potency [65]. When administered IV insulin aspart shows a similar safety profile with that of human insulin [109]. When further assessing its safety it was found that insulin aspart and soluble human insulin elicit the same counter regulatory and symptomatic responses to acute hypoglycaemia in patients with type I diabetes [110]. Insulin aspart has been shown to be absorbed twice as fast as human insulin and to reach maximum concentrations twice as high whereas its duration of action is shorter [111-113]. As expected, the postprandial

glucose control achieved with this analogue is superior to regular human insulin, whereas their bioavailability is comparable [112]. Mean postprandial glucose levels after any meal are lower, even when aspart is injected immediately before the meal and regular human insulin is administered 30 min before meals [114]. These results are consistent with those reported with the other short-acting analogue lispro but there is evidence that the improvement in postprandial control can be achieved without deterioration of late postprandial plasma glucose concentrations [115]. The expectation of lower rates of hypoglycaemia

Figure 7: Modifications of the Insulin Sequence in Insulin Glulisine.
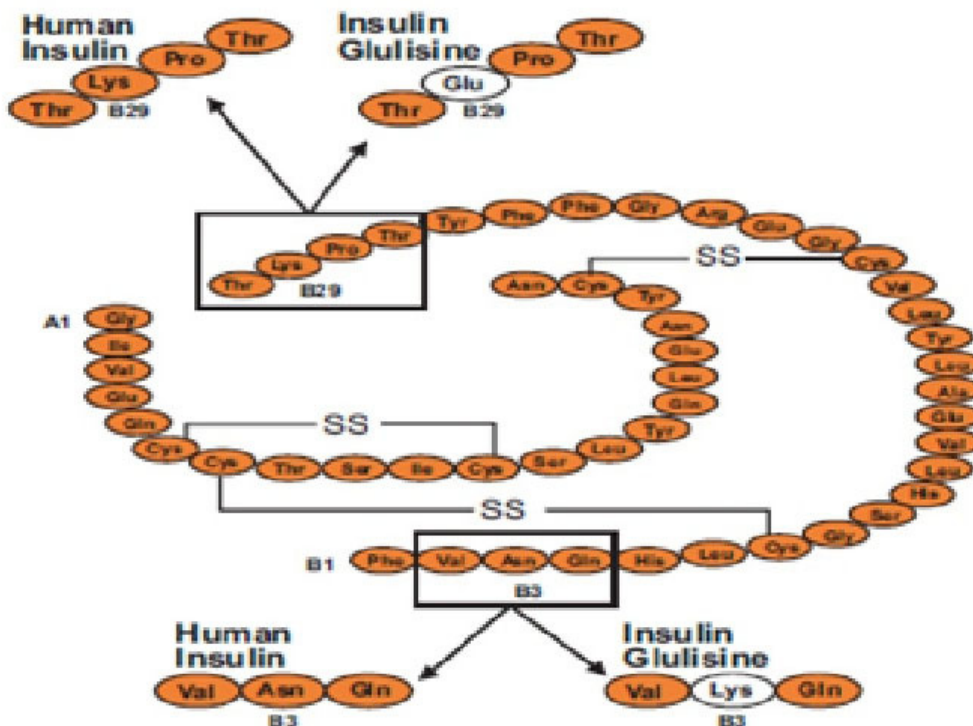
also seems to have been met with insulin aspart, as evidenced by a recent multicentre trial of type I diabetic patients, which showed more than a 50% reduction in major hypoglycaemic events compared with human insulin [115]. In a very interesting study with type I diabetics, it was found that, because of its rapid absorption, insulin aspart provided reasonable glucose control even when injected 15 min after the start of meals [116]. In the same study it was also found that after abdominal injections, aspart had a shorter duration of glucose lowering effect than after administration in the thigh or deltoid area [116]. The beneficial effects of insulin aspart have also been confirmed in type II diabetics [117] and in a paediatric population with type I diabetes [118]. Importantly this analogue retains its beneficial pharmacodynamics properties in a stable 30/70 premixed formulation, as it shows a significantly greater metabolic effect in the first 4 hrs with more rapid absorption and higher peak serum concentration than the 30/70 mixture of human insulin [119,120]. Because of its promising characteristics, studies are presently underway to evaluate long-term metabolic control with insulin aspart.

Insulin Detemir

In insulin detemir a C14 fatty acid side chain is attached to lysine at position B29 (**Figure 6**) in the molecule by an acylation reaction. It is an intermediate acting insulin analogue [121].

Novo Nordisk created insulin detemir and markets it under the trade name Levemir as a long-lasting insulin analogue for maintaining the basal level of insulin. The basal level of insulin may be maintained up to 20 hrs, but the time is clearly affected by the size of the injected dose. This insulin has a high affinity for serum albumin, increasing its duration of action [122, 123].

Glulisine Insulin

Glulisine is a newer rapid acting insulin analogue and the FDA-approved label states that it differs from regular human insulin by its rapid onset and shorter duration of action [56, 57].

In insulin glulisine the natural sequence of asparagine at position B3 and lysine at position B29 are substituted by lysine and glutamic acid respectively (**Figure 7**) [125, 126]. This structure of insulin glulisine affects not only self-association but also the isoelectric point, which is shifted lower (pH 5.1; human insulin, pH 5.5), which enhances its solubility at a physiologic pH. As a consequence unlike other insulin analogues that lack proline at B28, insulin glulisine is more likely to self-associate into dimers in the absence of ligands.

CONCLUSION

Diabetes mellitus is a metabolic disorder occurs due to insulin deficiency. The disease may leads to various metabolic complications and to treat these, r-insulin and its analogues are used.

In early years human insulin preparations are replaced by animal insulin's due to recombinant DNA technologies and advanced protein chemistry. Over the last decade, a number of insulin analogs were developed and to tested in the therapy of diabetes. The insulin lispro was one such short acting insulin anolog used clinically for the first time. This showed the improved glycemic control without an increase in hypoglycemic events. Later the availability of long-acting analogs such as insulin glraine replaced to short-acting analog insulin lispro. This helped to develop more individualized treatment strategies targeted to specific patient characteristics and to achieve further improvements in glycemic control. Combining different insulin analogs may even help to treat the multiple metabolic abnormalities of diabetics which are even beyond glucose metabolism.

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