A Review on Insulin Formulations for Diabetes Mellitus Therapy

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**Article History:**
Received on: 08 Jan 2020
Revised on: 11 Feb 2020
Accepted on: 25 Feb 2020

**Keywords:**
Basal insulin, Bolus insulin, Diabetes, Human insulin

**ABSTRACT**
The insulin discovery during the past century is considered as the major breakout in the health sector. It is considered as lifesaving medication for T1DM and T2DM. In the advancement of peptide chemistry, pharmacology, cell signalling and structural biology insulin has played a central role. Natural human insulin secreted from pancreatic cells maintains the glucose levels in blood. Later on, for the treatment of diabetes mellitus external insulin was developed by various means. However, imitating same actions by natural insulin by external insulin was difficult. Various insulin analogues developed (rapid acting, short acting, long acting, intermediate acting) different blood glucose lowering action profiles. The physiological post-prandial insulin response is not adequately reproduced by the pharmacokinetic profile of rapid-acting insulin. Before the meals, lyspro and aspart, fast acting analogues can be injected which produce faster and substantial insulin peak. As it is difficult to produce a basal insulin concentration for controlling pre-prandial blood glucose Level with available long acting analogues, insulin detemir and insulin glargine was synthesized. This article covers the detailed study of human insulin and various insulin analogues developed.

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ISSN: 0975-7538
DOI: [https://doi.org/10.26452/ijrps.v11i3.2527](https://doi.org/10.26452/ijrps.v11i3.2527)

**INTRODUCTION**
Diabetes mellitus is a chronic metabolic disorder which is characterised by higher glucose level in blood and later it can develop neuropathic complications. The development of this disease due to the defect in hormone namely insulin deficiency or insulin resistance. According to studies made by International Diabetes Federation (IDF) in 2017, approximately 425 million adults are living with diabetes. Diabetes caused death of almost 4 million people (International diabetes federation, n.d.). 90% of patients were reported with type 2 diabetes mellitus (T2DM) and remaining with juvenile diabetes or type 1 diabetes mellitus (T1DM) which is characterised by autoimmune destruction of β cells of pancreas which results in the complete loss of insulin production (Sanlioglu et al., 2008; Whiting et al., 2011). But T1DM is associated more of constant elevated ketoacidosis level.

Before 1920, treatment and diagnosis of people with diabetes mellitus was pitiable due to limited available choices, which resulted in increased mortality and morbidity mainly in children and young adult. During pre-insulin era diagnostic confirmation of diabetes meant ultimate coma and followed by death within 2 years after diagnosis (Vecchio et al., 2018). Physicians at that time used to manage disease by dietary modifications alone and affected individuals were restricted to diet which includes limited intake of carbohydrate in order to control blood glucose levels (Mazur, 2011; Zajac, 2010).
In early 1920s isolation and purification of insulin started which marked a milestone in the diabetes management.

**History of Insulin**

Diabetes was known for ages, from the account of symptoms related to diabetes and recommended treatment mentioned in the Ebers papyrus (around 1500 BC) from ancient Egypt. It was Apollonius of Memphis (230 BC), who used the term ‘diabetes’ for the first time. But even after two millennia, the cause for diabetes and the organ accountable for this condition was not explained. In 1889, Minkowski and Joseph von Mering in the laboratory of Oscar incubated a dog developed diabetes after pancreatectomy, with all typical symptoms of the disease (Lakhtakia, 2013). This helped to establish the major role of pancreas in the pathogenesis of diabetes. In future, Eduoard He’don, established that complete amputation of the pancreas was required for the development of diabetes (Hédon, 1893). Later He’don witnessed that grafting a small piece of pancreatic tissue after the removal of Pancreas lessened diabetes but it returned quickly on the tissue removal. Later in 1893 Gustave-Eduoard Laguesse suggested that the small clusters of ductless cells inside the pancreas— which was defined by Paul Langerhans in his doctoral thesis, and that he termed the Islets of Langerhans, may possibly the cause of the substance involved in glucose control (Zajac, 2010; Minkowski, 1989). It was Eugene Opie who confirmed the link between the islet cells and diabetes in 1901, and he connected islet cells degeneration to the diabetes genesis (Opie, 1901a,b). Afterwards, a number of investigators made attempts to isolate glucose lowering substance.

In 1921, young physicist Frederick Banting (1881-1941) and medical student Charles Best (1899-1978), working in Prof. McLeod’s laboratory at the University of Toronto, found the main link in a series of experiments initiated in 1916 by other researchers who conjectured the pancreatic secretion, already called “insulin,” capable of decreasing blood glucose levels. The young researchers extracted this pancreas extract that "cured" hyperglycemia in diabetic dogs (Banting et al., 1922), it was first delivered effectively to Leonard Thompson, a 14-year-old diabetic patient.

Marketing of animal insulin started by the company Lilly in 1923. By 1928, it was found that the hormone is a protein. Having identified thus, the researchers were after prolonging the action of Insulin produced by Banting and Bent lasted for less than six hours. It was Hagedorn, in 1936 who found that the duration of action can be increased by formulating insulin as suspension by addition of a basic protein, like protamine. He formulated and marketed first insulin “Isophane NPH” in 1946 by combining insulin and protamine in definite quantities at neutral pH (Yip et al., 2000). In Denmark, first lente insulin with retarded zinc and without protamine was developed in 1952 (Hallas-Mø, 1956).

**Human Insulin**

Insulin is a hormone that the pancreas normally produces. The insulin-producing cells of pancreas are known as β-cells. It has about 5808 Da molecular mass and is a dimer with two polypeptide chains, A and B, bound by two disulphide bonds. The polypeptide hormone consists of 51 residues of amino acids. The Figure 1 depicts the insulin with two chains A and B connected with disulphide bonds.

**Figure 1: Insulin**

At low concentrations insulin exist as monomer although it shows tendency to accumulate and form stable dimers at higher concentration, in aqueous solution at pH 2-8. In the presence of zinc ions, insulin form hexamers. As the chain A in hexamer consist of much of the polar surface it forms almost spherical structure of diameter 5 nm and a height of 3.5 nm.

Human insulin is synthetic insulin that mimics the original insulin produced in the body. Human insulin was completely synthesised in 1966 (Katsyannis, 1964) and approved for pharmaceutical use in 1982. Before the synthesis of human insulin, animal insulin usually porcine insulin was used. Discovery of human insulin itself was a milestone in the history of diabetes. Once it was identified that enough human insulin can be obtained from cadavers, the techniques for the insulin synthesis developed. By enzymatic method, synthetic insulin was obtained in which trypsin catalyses the replacement of alanine, in B30 position of pork insulin, with threonine. The insulin thus developed possesses an amino acid sequence similar to that the human and it was absolutely free of other hormones of pancreas (Morihara et al., 1979).

Genetic modification by the recombinant DNA tech-
nology enabled large scale synthesis of human insulin. DNA coding fragments for peptide chain A and B are separately introduced into plasmids and then to E. Coli for the in-vitro synthesis. The chains that are released into the medium was collected along with the bacterial proteins and with suitable disulphide bonds it was reassembled (Miller and Baxter, 1980; Chance et al., 1981). Contamination during various steps was avoided by using plasmids formed with genetic coding material for proinsulin molecule. Proinsulin, which is deprived of connecting peptide is isolated from culture medium (Johnson, 1982). Another technique for the production of biosynthetic insulin involves the yeast Saccharomyces cerevisiae. A purified DNA that codes the synthesis of a single chain precursor is inserted into yeast’s plasmid. During fermentation, yeast produce a protein, which is collected from the culture medium and followed by centrifugation and crystallisation for the isolation. It was then transformed to insulin ester by trypsin transpeptidation and lastly hydrolysed. Highly pure insulin can be produced by this method (Galloway et al., 1992; Lenaghan et al., 2011).

Nowadays, different human insulins exist in market (Table 1). Insulin that acts quickly (rapid acting) includes insulin lispro, insulin aspart. Short acting insulin is regular insulin whereas lente and NPH comes in intermediate acting and Ultra Lente is long acting. The regular insulin formulation is a clear colourless neutral pH solution (7-7.8). Meta-cresol is used as a preservative, glycerol as a tonic stabilizer. The prevalent quaternary form of pharmacological insulin is hexamers made stable by zinc ions; other structures include dimers and tetramers. In the vial and in the tissue (where it is injected), the molecule appears to accumulate. The hexamers must be converted to monomer at the subcutaneous injection site for the absorption of insulin. For this reason, after a delay period about 30 minutes after injection, regular insulin reaches the general circulation. It hits the plasma maximum at 2-4 hours and lasts 6 hours.

Protamine, a protein obtained from fish sperm delays the absorption of NPH. Isophane-NPH insulin is a white orthorhombic crystalline suspension containing 0.9 protamine molecules and 2 zinc atoms per hexamer (Yip et al., 2000). The interaction between dimers and hexamers are regulated by protamine. The water, which is used as vehicle is buffered at a pH of 6.9-7.5. As preservatives, phenol or metacresol are added. As insulin crystals are insoluble in water, it shows tendency to precipitate at the bottom of the vial. So it is necessary to shake it before use for re-suspending them (Jehle et al., 1999). NPH is a longer-acting insulin, its blood absorption starts 1.5 hours after subcutaneous injection. It attains maximum plasma concentration at 4 to 12 hours and within 24 hours its action disappears. Nevertheless, the “tail” is relatively ineffective. Although NPH has the most frequent absorption of all intermediate-and long-acting insulins, there is high inter-and intra-individual variability.

In Lente insulin, absorption is delayed by the precipitating hormones with salts of zinc. When the insulin-related molar ratio of zinc ions is greater than one, insulin solubility in the neutral solvent is reduced (Hallas-Mo, 1956). When insulin solution is adjusted to pH 7.4 with zinc ions amorphous precipitate is produced. Upon subcutaneous injection, it slows absorption. This type of insulin is called as insulin semilente.

Long-acting Ultralente insulin is an aqueous suspension at neutral pH of zinc-insulin crystals. It has an onset of action after 4 hrs and reaches a modest but excessive maximum plasma concentration at 7 hours, and supports blood insulin levels of about 8 to 20 h; this inconsistently imitates endogenous basal secretion. In addition, its absorption is completely irregular (Binder et al., 1984) and cannot be mixed in the syringe with regular insulin due to the excessive delay in the action of the syringe. Various insulin analogues available in market are given in the Table 1.

In 1980s most of the diabetic patients were provided with combination of regular and NPH or zinc-based insulin (in premixed form or mixed by patients), with an intake of twice daily before food. The DCCT (Diabetic Control and Complication Trial) proved that tight glycaemic control resulted from intensive insulin therapy prevented T1DM related microvascular complications (Nathan and Group, 2014). Thus an intensive insulin therapy was established using regular insulin before food and basal insulin administrated at bed time and are regarded as gold standard in the T1DM therapy. The limitations such as increased risk of hypoglycaemia in patients underwent insulin replacement therapy, especially human insulin were highlighted by DCCT. Furthermore, 30% higher risk of gaining weight is shown by intensively treated patients than non-intensively treated patients. The lack of negative feedback on release of insulin from subcutaneous depots after insulin injection or discrepancy between action profiles of human insulin preparation and basal level can be the reason for the adverse effects of intensive insulin therapy.

**Insulin analogues**
Table 1: Insulin analogues

| Types of insulin | Onset of action | Peak(hours) | Duration(hours) |
|------------------|-----------------|-------------|-----------------|
| Rapid acting     | 5-15 min        | 0.5-2       | 2-4             |
| Insulin lispro   | 5-10 mins       | 1-3         | 3-5             |
| Insulin aspart   |                 |             |                 |
| Short acting     | $\frac{1}{2}$ - 1 hr | 2-3       | 5-8             |
| Regular          |                 |             |                 |
| Medium acting    | 2-4 hrs         | 4-12        | 12-18           |
| NPH, lente       |                 |             |                 |
| Long acting      | 4-6 hrs         | Unpredictable | 24+            |
| Ultralente       | 1.1 hrs.        | none        | 24              |
| Insulin glargine |                 |             |                 |

### Regular insulin

Regular insulin also has a similar structure like naturally produced human insulin from pancreatic β-cells. It has six monomers of insulin placed around a zinc ion to form a hexamer. Each monomer contains two chains: chain A and chain B linked together by two disulphide bonds. It also has an additional disulphide bridge between two amino acids in the chain A. As soon as this reaches bloodstream upon injection, hexamers dissociate into monomer and this interacts with the insulin receptors on target tissues. This shows that regular insulin administered by intravenous route has an immediate glucose lowering effect. However, before resorption into bloodstream can occur, the dissociation of hexamer into monomer should happen, when injected subcutaneously. Hence, depending upon several factors like site of injection, flow of blood and body temperature, there can be a delay in onset of glucose lowering action with subcutaneous administration of regular insulin.

### Zinc and NPH insulins

The inclusion of zinc or protamine to regular insulin leads to the formation of lumps as the insulin is associated to these substances, causing their action profiles to be prolonged. The variance in their action profile is this insulin’s major throwback. Therefore, insulin resuspension in the vial is required prior to injection. But there is still variation in the exclusively controlled laboratory condition resuspension (Heise et al., 2004). During subcutaneous injection, from zinc or protamine depot regular insulin hexamers are released in a controlled way for several hours. This results in a highly inconstant insulin release action profiles with a range of a few hours to over 24hours’ duration of action. Variation in action profile, the strength of the insulin action and duration of action, with peak levels of release soon after administration are seen in these insulin.

Inability to cover the basal insulin needs for 24h in patients constitute the major limitation of these insulins (Heise and Pieber, 2007; Lucidi et al., 2011).

### Rapid-acting insulin analogues

The rapid acting insulin are formulated as unstable insulin hexamers so that it can easily get converted to dimers and monomers in solutions. This results in the rapid movement of insulin into bloodstream after administration by subcutaneous injection than human regular insulin. This faster action profile helps in reducing the time between meal and injection, thus offers a improved match between the insulin-action profile and the glucose excursion caused by meals, than the human regular insulin (Brange et al., 1990). Insulin lispro, Insulin aspart and Insulin glulisine are three rapid acting insulins available for clinical use.

### Insulin lispro

There is only a small difference in the molecular structure of insulin lispro from that of normal human insulin, the order of proline and lysine in the B chain at 28 and 29 residues respectively. Hexamerization of the insulin is destabilised by this alteration and this enables faster dissociation into dimer and monomer, which enables the faster uptake by blood vessels and rapid action of insulin (Home, 2012). It shows a peak concentration within 1 hour of administration.

### Insulin aspart

There is a small molecular structural difference in insulin aspart from that of human regular insulin. The proline in 28th position of B chain of regular insulin is replaced by aspartic acid in insulin aspart (Home, 2012; Lindholm et al., 1999; Home et al., 1999). The pharmaceutical formulation of insulin aspart contains m-cresol, zinc, glycercine, phenol and a buffer, disodium hydrogen phosphate (Home, 2012). The insulin aspart have similar
PK-PD action profile as that of insulin lispro (Lindholm et al., 1999), and most studies shows that both insulin lispro and insulin aspart shows similar glucose lowering effects with no difference in the time to maximum concentration of insulin (Home, 2012; Plank et al., 2002; Homko et al., 2003). So that insulin aspart can be administrated 15 mins before the start of a meal (Lindholm et al., 1999; Home, 2012). The postprandial glucose achieved by insulin aspart is remarkably lower than that of human regular (Bartolo et al., 2008; Bode et al., 2002; Dreyer et al., 2005; Home et al., 2000; Raskin et al., 2000).

Insulin glulisine

In Insulin glulisine, in contrast to aspargine and lysine at residue 3 and residue 29 (chain B) in regular human insulin, lysine and glutamic acids are placed respectively (Home, 2012; Becker et al., 2005). The insulin glulisine formulation comprises polysorbate 20 as a substitute of zinc (Becker et al., 2005). More frequent issues like clotting and catheter obstructions are found with use of insulin glulisine. Hence, insulin aspart and insulin lispro are favored over insulin glulisine for use in subcutaneous pump catheters as they are stable (Kerr et al., 2013). Insulin glulisine have a slightly faster onset of action than the other analogues (Becker et al., 2005; Heise et al., 2007; Arnolds et al., 2010). In patients with obesity, the faster onset of action of insulin glulisine was evident (Luzio et al., 2008; Bolli et al., 2011). The rapid onset of action can be attributable to the zinc-free formulation of insulin glulisine, as zinc in insulin aspart and insulin lispro delays the absorption and action of insulin by slowing down dissociation of hexamer to monomer after injection (Heise et al., 2007).

Long-acting insulin analogs

Insulin glargine and insulin detemir (the first long-acting insulin analogues) were formulated to offer more stable and longer basal insulin-action profiles better. The intend of long acting insulin is to give 24h action coverage to improve patient compliance.

Insulin glargine

It was the first approved basal insulin analogue for clin i (Lepore et al., 2000) cal use. The prolonged action is due to its precipitation in the subcutaneous tissue. Its precipitation leads to aggregate formation and induce long term release. Insulin glargine is soluble in acidic pH and precipitate formation occurs only in neutral pH (Porcellati et al., 2007). Hence, to maintain neutral pH two arginine molecules were introduced to the B chain amino terminus. Additionally, asparagine at residue 21 of A chain is substituted with glycine. The duration of insulin glargine action under single dose is 22-24 h. It has a less variable action profile than NPH insulin. This decreases the risk of hypoglycaemia particularly nocturnal hypoglycaemia (Lepore et al., 2000).

Insulin detemir

Insulin detemir is a basal insulin which is soluble in neutral pH. A 14-carbon myristoyl fatty acid was added to lysine at residue 29 of the B chain in residue 30 of the B chain. It promotes the self-association in tissue and bloodstream of insulin detemir molecules into dihexamers at the injection site and reversible albumin binding. Insulin detemir’s prolonged action is attributed to hexamer formation and binding of albumin. The required dose of insulin detemir is slightly higher than the insulin from the NPH.

CONCLUSION

Compared to NPH insulin, long-acting insulin analogs offers extended duration of action approximately 24 hours and also it is able to reproduce the serum concentration. These characteristics imitates the steady and slow basal release of insulin for correction of slight variations in glucose level. Rapid acting insulins like insulin lispro and insulin aspart get absorbed quickly and attains peak faster. However, rapid acting insulin have a duration of action shorter when compared to naturally produced human insulin. These are the characteristics of rapid acting insulin that imitate the insulin rush in prandial phase in response to glucose excursions. The glucose lowering capabilities are more and faster with premixed insulin analogs than premixed human analogs. The risk hypoglycaemia is lower with long acting analogs than rapid acting analogs and NPH. When compared with normal human insulin, premixed insulin shows improved glycemic control on appropriate dosing especially for post-prandial condition. Because of relatively reduced risk of hypoglycaemia, greater flexibility in dosing and improved patient compliance insulin analogs are preferred. Even though treatment using insulin analogs offers enhanced and stable glycemic control, improvements are needed in areas like frequency of dosing, nocturnal glycemic control and rapid prandial actions to become similar to the natural human insulin functioning.

Diabetes incidence are increasing day by day, so more effective therapies of insulin is required. Adequate control of blood glucose with simple and appropriate dose regimen and without any hypoglycaemic episode is the major concern regarding the insulin therapies.

Conflict of Interest
None.

Funding Support
None.

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