Review Article

A 100 Years Journey of Insulin

Rakesh Chandra Chaurasia[1]

The discovery of insulin and the journey of evolution has revolutionized the saga over the last 100 years. Diabetis Millitus (DM) is a worldwide health issue that causes serious disability. The complications like renal failure, amputation, and loss of eyesight are huge social burdens and their economic cost are also enormous. Being a central etiological factor Insulin™ is a mode of therapy. There are ranges of conventional to newer insulin. The latest insulin has come through various stages after the advent of recombinant DNA technology. The native insulin molecule is modified via substitution, replacement or addition of some amino acids to form Insulin analogs™ (designer insulin) along with a change in biological activity and their pharmacological profile. The more recent inclusion is smart insulin. Other newer insulin is under various phases of a clinical trial. This article highlights a hundred years of anniversary pertinent to insulin along with significant milestones in medical history.

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Diabetes Mellitus (DM) is mentioned as 'Madhumeha' (honey urine) in ancient Indian texts - Charaka & Sushruta Samhita. Madhu means sweetness like honey and meha means to flow. The term Diabetes Mellitus was introduced by Thomas Willis (a British physician-1621-75) to clinically differentiate this from diabetes insipidus.

Diabetes Mellitus (DM) is a global disease that results in significant morbidity and mortality. These days, developed nations have witnessed an explosive increase in the incidence of DM, predominately related to lifestyle changes. International Diabetes Federation estimated that more than 500 million people Worldwide are affected and its prevalence is expected to more than double by the end of the third decade of this century[1].

Diabetes Mellitus is caused by a decrease in the circulating concentration of insulin ie, insulin deficiency and a decrease in the response of peripheral tissue to insulin ie, Insulin resistance. These abnormalities lead to alterations in the metabolism of carbohydrates, amino acids, lipids and ketones. Clinically, most patients are classified as either Type -1 DM or Type - 2 DM. Other causes are gestational (GDM) and secondary. Maturity-onset Diabetes of Youth (MODY) is another alarming variety that incidence is growing so fast. The central feature of diabetes syndrome is hyperglycemia, responsible for the development of complications. Treatments directed to maintenance of normal blood glucose level. Regular exercise, adequate diet, and average body weight control blood parameters and counteract several co-morbid conditions[2].

Editor's Comment :

- Insulin was discovered 100 years ago and is still the mainstay of almost all Type 1 and non-responding Type 2 Diabetes Mellitus.
- Originated from animals as conventional bovine and porcine insulin, now the era is diverted toward biosynthetic human insulin, then designer insulin and most recently under trials innovative smarts insulin.
- Insulin is the only miracle medicinal molecule linked with the maximum number of prestigious Nobel Prizes.
- For community awareness 14th November, the birth anniversary of Banting is designated as - World Diabetes Day.

The evolution of insulin therapy over the past 100 years since the discovery of insulin is testimony to the biomedical bench-to-bedside process. Insulin is the mainstay of treatment of virtually all types of DM-1 and non-responding Type-2 DM. These days, numbers of insulin preparations are available viz conventional, purified, human and more recent insulin analogs. Such analogs offer an advantage over other insulin due to the convenience of dosing at mealtime and their more physiological pharmaco-kinetic profile, which may allow patients to achieve better glycemic control. These analogs are popularly known as 'designer insulin' due to changes in the design pattern of native insulin[3].

Historical Scenario :

The history of insulin discovery is a saga of trials, failure, hope, discovery and rediscovery. In 1886, Paul Langerhans, a German medical student, noted that the pancreas contained two distinct groups of cells- the acinar cells which secrets digestive enzymes, and cells that are clustered in the island (islets of

[1]MD, Professor, Department of Pharmacology, MLN Medical College, Prayagraj, Uttar Pradesh 211002 and Corresponding Author

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Langerhans], which he suggested to serve as second function(?). This comes to light in 1889 when Minkowski and Von-Mering showed that pancreatectomized dogs exhibit a syndrome similar to DM in humans.

There were numerous attempts to extract the pancreatic substance (insulin) responsible for blood glucose regulation. In 1900, Gurg-Zuellzer in Berlin attempted to treat a dying DM patient with an extract of the pancreas. In 1911, EL Scott, a student at Chicago University attempted to isolate an active principle and treated several diabetic dogs with encouraging results. In between 1916-20, the Romanian physiologist Nicolas Paulesco found that injection of pancreatic extract reduces urinary sugar and ketones in diabetic dogs but their significance appreciated after several years.

Frederick Banting, a young Canadian surgeon together with Charles Best, a fourth-year medical student, successfully obtain a pancreatic extract after degeneration of acinar tissue by ligating the pancreatic duct, which decreased the blood glucose concentration in diabetic dogs. Leonard Thompon, 14 years was the first patient to receive the acinar extracts prepared by Banting and Best at Toronto Hospital with blood glucose 500 mg/dl and demonstrated marked clinical improvement. The remarkable work on insulin came with lots of hope for diabetics. That news spread in most parts of the world like wildfire[4].

Insulin & Nobel Prizes :

Banting and Best faced difficulty to obtain active extract reproducibly. This led to the greater involvement of Macleod, a professor of physiology at Toronto and JB Collip, (a chemist with expertise in the extraction and purification of epinephrine). The stable extract was eventually obtained from animal sources. The prestigious prize in Medicine and Physiology was awarded to Banting and McLeod in 1923. Banting announced that he would share his prize with Best. MacLeod did the same with Collip. Frederick Sanger determine the primary structure of insulin and was awarded Nobel Prize in 1969. Dorothy Hodgkin gets Nobel Prize to determine the tertiary structure by means of X-ray diffraction studies[5]. Insulin is the only miracle molecule concerned with a maximum number of Nobel Prizes. The 14th of November – the birth anniversary of Banting is celebrated as 'Diabetes Awareness Day'[6].

Insulin Structure :

Frederick Banting and Charles Best first extracted insulin in 1921. Sanger established the amino-acid sequence in 1960. Hodgkin and co-workers elucidated three- dimensional structure of insulin in 1972.

Insulin consists of two peptide chains A and B, interconnected with two intra-subunit and one inter-subunit disulfide bridges at the A7-B7 and A20-B19 position of amino acid in the chain. A-chain is composed of 21 amino acids while a B-chain has 30 amino-acid residues. Therefore, human insulin has a total of 51 amino acids with a molecular mass of approximately 5734 Daltons[7]. Insulin exists as a monomer, dimer and hexamer. The monomer is the biologically active form of insulin.

Conventional to Newer Insulin — A Long Journey :

The clinical introduction of Insulin, almost a century ago in 1921 has revolutionized the treatment of diabetes. Initially, insulin was manufactured from bovine and porcine sources. Bovine insulin differs from two amino acids while porcine insulin by one amino acid[4]. The conventional commercially available preparation contained some antigenic impurities that act as an allergen to some users. In 1983. Human insulin is produced by recombinant DNA technology using special strains of E coli or yeast. Insulin allergy, lipodystrophy, and resistance were almost eliminated with the development of biosynthetic human insulin almost. The present-day approach is to deliver insulin in a physiological manner, which will refer to a rapid release at mealtime and the low stable release of insulin in between meals. These initiate the making of new designs of insulin from existing ones.

Need of Advanced Insulin — Why ?

The value of glycemic control in the prevention of complications was emphasized in landmark clinical trials in 1990 (United Kingdom Prospective Diabetic Study UKPDS) and (Diabetic Control and Complication Trial DCCT). The scientific evidence of the trial provides a basic need for newer insulin formulation, which could mimic closely both mealtime and the basal component of endogenous insulin secretion. This urges the development of insulin analogs. Because of the change in the design of native insulin such newer preparations are popularly known as 'designer insulin'. With the advent of genetic engineering, human insulin is now produced by recombinant DNA technology using a special strain of E.coli and yeast. Modification of amino-acid sequences of human insulin has produced different designs with different pharmacological profiles[8]. Other insulins are under clinical trial to enhance patient compliance.

Basic Issue in Designing :

Any amino–acid moieties are not utilized due to adverse effects or carcinogenic potential. As general

amino acids, no 25-30 of the B-chain is most suitable to target for design. Thus, only a few molecules such as lysine, proline, etc are receptive to an effective change and are the sites where the current engineering trends for newer insulin analogs focused. The analog should mimic the physiological profile, pharmacologically better than the existing one along with a better safety profile. Insulin analogs provide the overall view of the structure-activity-relationship between insulin biochemistry and action.

Therefore, insulin structure thus provides many options for the modification of newer variables[9].

Newer Insulins in Clinical Practice :

Newer insulin analogs differ from native insulin in amino-acid sequence and are obtained by a novel technique of genetic engineering by induction and deletion in A and B chains of the native form ie, by modification in the design of insulin. Therefore, designer insulin is identical to human insulin except for the positions of some amino acids that are changed. The designer insulin is classified according to onset and duration of action into the following category.

1 - Rapid Acting Insulin —

- Insulin Lispro
- Insulin Aspart
- Insulin Glulisine

2 - Long Acting Insulin —

- Insulin Glargine
- Insulin Detemir
- Insulin Degludec

Lispro was the first short-acting insulin analog approved in 1996, followed by aspart in 2000 and glulisine in 2004. On the other hand, glargine was approved in 2000 and detemir in 2005. The most recent ultra-long-acting degludec was approved in 2015. After structural modification, the solubility, onset and duration of their biological action get changed[10].

Apart from rapid and long-acting designer forms, biphasic ones, better known as intermediate-acting insulin are also available. They provide both mealtime and basal coverage in a single preparation. Premix biphasic insulin analogs are combinations in various proportions, Insulin degludac & insulin aspart is the first analog combination that contains both long-acting and rapid-acting insulin. It is a unique combination that provides a stable insulin action over a 24-hour period[11]. Brief descriptions of currently available preparation are mentioned :

Insulin Lispro — The amino-acid lysine and proline near the carboxyl terminal of the B-chain has reversed. Proline at B28 has been moved to B29 and lysine from B20 has been moved to B28. Thus, normally occurring Pro-Lys sequence at B28 and B29 is reversed to Lys-Pro. To enhance shelf life, insulin lispro has stabilized into hexamer and dissociated into monomer almost instantaneously following injection. This property results in characteristic rapid absorption with an onset of action within 5-15 minutes and a shorter duration of action ie, 3-5 hours.

Insulin Aspart — This was created by the substitution of neutral proline at B28 with negatively charged aspartic acid. This modification reduces the normal proline B28 and glycine B23 monomer-monomer interaction, inhibiting insulin self-aggregation and rapidly breaking into monomer after injection, resulting in ultimate rapid onset and shorter duration of action.

Insulin Glulisine — Glutamic acid replace lysine at B29 and lysine replace asparaginase at B3. This will cause a reduction in self-association and rapid dissociation into active monomer. The net result is the short onset and duration of action.

Insulin Glargine — This is the first long active analog produced by alteration in both A and B chains. Two arginine residues are added to the carboxyl terminus of the B chain and asparagine in A21 is replaced with glycine. It is a clear solution at an acidic pH. This pH stabilizes the insulin hexamer and forms an amorphous micro-precipitate. From these crystalline depots, insulin is slowly released resulting in prolonged sustained and predictable absorption patterns. Insulin Glargine has a slow onset of action and achieved maximum effect after 4-5 hours with maximum activity maintained for 12-24 hours or longer. It has 6-7 fold greater binding than native insulin-to-insulin receptors. Glargine insulin is administered at any time during the day with equivalent efficacy, usually given once daily. Although some very insulin-sensitive individuals will benefit from split dosing like twice a day. There are two generations of Glargine insulin on the basis of the concentration of units of insulin per ml First G – 100U/ml (introduced in 2000), and Second G- 300U/ml (introduced in 2015).

Insulin Detemir — Detemir is obtained by the removal of threonine at position B10 of 14-carbon myristoyl fatty acid at the epsilon group of lysine at B 29. The addition of fatty acid stabilizes and increases the solubility of insulin. This modification has increased its association with insulin to albumin and imparted the molecule with reversible albumin binding capacity, thereby prolonging the duration of action through slow dissociation from albumin. Insulin detemir is soluble at a neutral pH of 7 which overcomes the problem of precipitation and crystallization. This insulin is more slowly absorbed and its effect last for more than 24

hours. Therefore insulin detemir should be given once daily at a fixed time[12].

Insulin Degludec — Most recent inclusion in the list of long-acting insulin is ultra-long-acting insulin named degludec. A single amino acid threonine is deleted at the B-30 position and is conjugated to hexadecanedioic acid via gamma L glutamyl spacer linker at amino acid lysine at position B-29. This structure allows the formation of multi-hexamer as a depot in subcutaneous tissues that result in the slow release of monomers into systemic circulation thus there is no peak activity. It has a duration of action that lasts up to 42 hours, the highest among other long-acting preparation, Degludac can be given thrice weekly and is commonly called as ultra long-acting insulin[13].

Pharmacological Profiles :

The designer insulin mimics the physiological pattern. These analogs bind with the insulin receptor and act like native insulin. They display longer non-peaking profiles with better glycemic control.

Rapid-acting insulin analogs offer more flexible treatment regimens. These bear a close resemblance to normal mealtime insulin and therefore improve post-prandial glycemic control and will reduce the risk of hypoglycemia. This has the additional benefit of allowing insulin to be taken immediately before a meal. These analogs retain their structure in the monomeric or dimeric configuration on subcutaneous injection and are thus absorbed three times more rapidly than usual insulin. As a result, there is a rapid increase in plasma insulin levels and early onset of hypoglycemic action. These analogs can be injected just before or just after mealtime ie, 'shoot and eat.' Some formulations are also suitable for intravenous administration, much better via an insulin pump[14].

Long-acting insulin analogs have a slow onset and prolonged action. They provide a low basal concentration of insulin continuously throughout the day. Increased stability, less variability and a more selective action do help treatment strategies to achieve far superior glycemic control, therefore, preventing complications. They have flat and prolonged hypoglycemic effects ie, no peak. These analogs exhibit a longer action of up to 24 hours and should be injected once daily. The ultra-long-acting insulin can be given thrice a week[13].

As biphasic insulin analog is a combination of both long-acting and rapid-acting insulin in various proportions, thus they provide mealtime and basal coverage in a single preparation[15].

Insulin Delivery Devices :

Being a peptide hormone, insulin gets destroyed by gastric acid if taken orally while absorption from the parenteral routes is not reliable but also inconvenient to the patient as for self-administration is concerned[16]. Considering all parameters and ease of self-administration, the subcutaneous route is the most widely accepted way of insulin administration[17]. There have been landmarks in the insulin delivery system starting from the syringe (1924) to the most current, precise and accurate means. Common insulin delivery system includes insulin syringes, insulin pen, jet injector, and insulin infusion port/ pump. Recent innovations are under trial, a few of them are continuous intraperitoneal insulin infusion, patch pumps, oral insulin, artificial pancreas and insulin inhaler[18].

Future of Insulins :

Once weekly Insulin – Design modification of insulin icodec include three amino acid substitutions (one at A chain, two at B chain) and a C20 fatty diacid side chain that is attached through a hydrophilic linker at B-29 K. These changes yield strong yet reversible binding to albumin, reduced enzymatic degradation, and slower receptor-mediated clearance, resulting in a half-life of approximately 196 hours.

Hepato preferential Insulin — Insulin peglispro is a pegylated basal insulin that has a rapid effect in suppressing hepatic glucose production and a lesser delayed effect on peripheral glucose uptake. The hepatic preferential effect has been attributed to its large molecular size.

Inhaled Insulin — A formulation inhaled as a dry powder with a shorter duration of action about 3 hours (afrezza) and 6 hours(exubera) .

Oral Insulin — Isosema, tregopil,oramed, and lyn are oral insulin, under clinical trial.

Glucose Responsive Insulin — Also called 'smart insulin' because of the dynamic regulation of insulin secretion. As exogenous insulin contains a glucose-responsive moiety. This sensor would release insulin when blood glucose concentration exceeds the normal range but not when they are within target[19].

Newer Adjunctive Modalities :

Incretin is a group of hormones that includes Glucagon-like Peptide (GLP-1) and Glucose-dependent Insulinotropic Polypeptide (GIP-1). In spite of a potent insulinotropic incretin, GLP-1 has a short life span and is quickly metabolized by Di Peptidyl Peptidase IV (DPP-IV) enzyme within 1 to 2 minutes. Incretin action can be enhanced by degradation resistant GLP-1 receptor agonists (incretin mimetics) and by inhibitors

of dipeptidyl peptidase iv. activity [incretin enhancers]. Currently, available incretin mimetic are injectable agents and includes exenatide, liraglutide, pramlintide, etc. Being a polypeptide, exenatide and another agent must be administered by the subcutaneous route. These agents are used as add-on drugs with metformin or sulfonylurea, with insulin. The usual side effects are nausea, vomiting, diarrhea, and really fatal narcotizing hemorrhagic pancreatitis[20].

Incretins are rapidly metabolized by the enzyme Di Peptidyl Peptidase –IV (DPP-IV) Inhibitors, Their action can be enhanced by inhibiting enzymes, ie, by the use of specific enzyme inhibitors (gliptins). The DPP-1V inhibitors include sitagliptin, saxagliptin, vildagliptin and linagliptin. The most common adverse effects are headaches and nasopharyngitis. Gliptins are used in combination with metformin and sulfonylurea[21].

Amylin is another polypeptide hormone co-secreted with insulin. It also inhibits glucagon release and suppresses appetite. Pramlintide is an available amylin mimetic representative used subcutaneously as an adjunct to insulin, by separate injection[22].

Sodium-glucose Transport Protein (SGLT2) is responsible for glucose absorption from the kidney. Its inhibitor reduces blood glucose by inhibiting renal glucose re-absorption. Numbers of agents are under clinical trial. Insulin-like Growth Factor (IGF-1) is tried in severe insulin resistance. Aldose reductase inhibitors inhibit the formation of polyol from sugar thereby reducing the thickening of the basement membrane. Glucosamino-glycane (CAG) has some preventive role in diabetic nephropathy. Cannabinoids receptor antagonists, lipase inhibitors, and T-cell inactivators are under trial as diabetic preventive agents[23].

Conclusion

2021 is marked as the centenary year of Insulin discovery. It was ground-breaking innovation in the history of diabetes care. Diabetes Mellitus is a global health problem of grave morbidity and mortality. Insulin was discovered 100 years back and is still the only documented scientific way of medical hope. During that period the number of preparations is introduced one by one with a definitive advantage over the previous one. Although insulin has been available for several years, major advances have been made over the past few decades after the advent in advance of biotechnology, especially genetic engineering. The most recent approach is to deliver insulin in a physiological manner. This is only possible after the advent of certain analogs, the most popular among them is designer insulin. These are well tolerated and closely mimic physiological patterns to achieve better metabolic control. 1921 to 2021 has witnessed the milestones of insulin innovation from conventional animal origin to genetically engineered human insulin, designer as well as smart insulin. Exactly 100 years before that in 1923, Nobel Prize was awarded to stalwarts for their breakthrough invention.

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Ethical Consideration – Not required

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