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Quest for superior insulins

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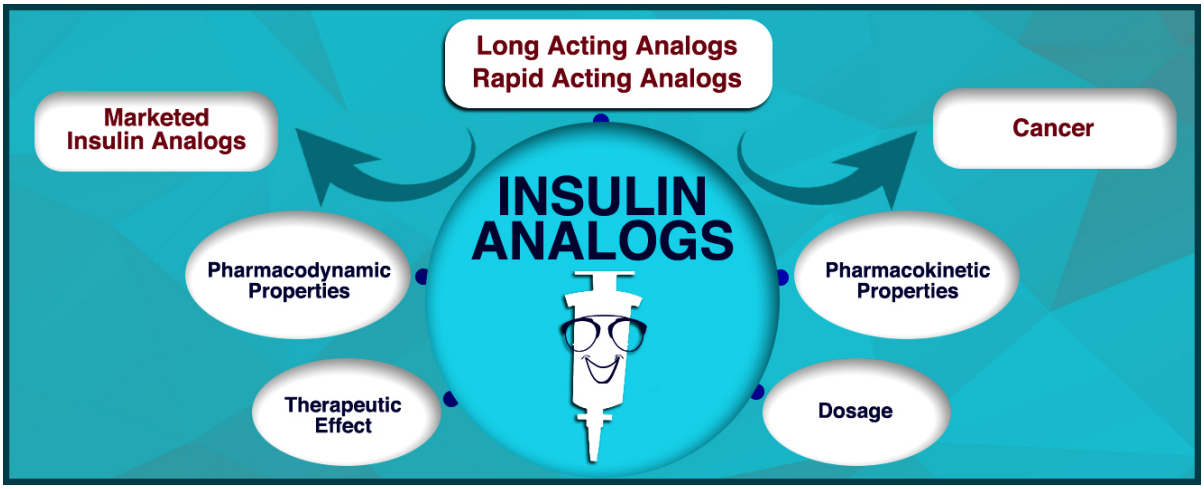
ABBREVATIONS:

T1DM - Type 1 Diabetes Miletus
NPH - Neutral Protamine Hagedorn
FPG - Fasting Plasma Glucose
TI - Technospheres Insulin
GRI - Glucose Responsive Insulin
FDKP - Fumaryl Diketopiperazine Powder
IGF-1R - Insulin Like Growth Factor 1 Receptor

ABSTRACT:

Chronic metabolic illnesses, like diabetes mellitus, have become diseases that harm human health and are now one of the most critical public health problems in almost half a century, as a result of societal development and lifestyle changes. For decades, conventional insulin therapy has been playing a significant role while treating millions of patients around the globe. Unfortunately, despite breakthroughs in DNA recombinant technology and pharmacotherapy, these therapeutic goals are rarely met, and results have not improved significantly. The low effectiveness rate of insulin treatment is becoming recognized as a result of intra-individual and inter-individual differences in insulin needs. Thus, our review focuses on finding superior insulin derivatives to treat diabetes more effectively and efficiently. Insulin analogs hold the potential to overcome the limitations of conventional insulin. We have taken a deep dive into rapid acting and long-acting analogs by discussing their pharmacokinetics, pharmacodynamics, dosage and therapeutic efficacy. But their safety profile has been questioned several times and therefore we have thrown some light on the current innovation trends that are being scrutinized.

VISUAL ABSTRACT:



KEY WORDS: Insulin Analogs, Type 1 diabetes, cancer, marketed insulin, rapid acting analogs, long-acting analogs, smart insulin, oral insulin, inhaled insulin

1. INTRODUCTION:

The 'ERA' of Diabetes has just started and have always had an upward projectile, it is projected that by 2030 there would be around 600 million diabetic patients [1]. Diabetes being one of the oldest and genetic disease, has its root across all over the world, but "with every sword there always comes a shield." Insulin came out as one of the best treatments for diabetes [2]. Diabetes is caused when the cells (producing Insulin) in the pancreas gets destroyed because of autoimmune destruction hence the glucose level increases, so to control it there was a need to provide insulin through an external source [1]. Insulin was discovered by Banting, McLeod, Collip, and Best in 1921. J B Collip produced a successful, contaminant-free extract of insulin from chilled beef pancreas [3]. Patients responded positively to this new series of injections- their glycosuria, ketonuria disappeared, and blood glucose levels dropped to normal [3].The year 2021 marked 100 years of insulin discovery and many modifications came along the way, by the 1990's Insulin analogs came to the market and were preferred because of the duration of action according to the need, consistent effect, Pharmacoeconomic advantage, and many other factors [4]. Analog were divided into 3 parts according to the duration: Long-acting, rapid-acting, and intermediate, "sweet would not always be served, sometimes Bitter things would always be there", Insulin analogs also possess side effect and some may be there for a long-term; the theory that it can cause Cancer is not proved and is still controversial, but people sensitive about their weight would prefer Insulin analogs over Human Insulin since there is no significant gain [5].

2. BASIC BIOLOGY BEHIND INSULIN SECRETION:

Beta cells of pancreas secrete insulin in response to various stimuli such as glucose(major), sulfonylureas, arginine, etc. Beta cells pick up glucose through GLUT-2 receptors. In beta cell, glucokinase oxidizes glucose. If glucose concentration is low, insulin is not released and Beta cell membrane is negatively polarized. As, the glucose concentration in blood increases, glucose uptake by GLUT increases leading to depolarization of membrane and Ca2+ voltage gated channels are opened. This exocytosis due to Ca2+ influx, leads to release of insulin [6]. Recombinant DNA Technology is used to produce human insulin. This involves isolation and introduction of human insulin or proinsulin gene into E. coli.

Type 1 diabetes is an autoimmune disease caused due to destruction of Beta cells of pancreas and hence resulting in reduced insulin production. Insulin therapy controls the blood glucose levels and also is thought to be the reason for beta-cell recovery because hyper-glycemia may decrease insulin response6. C-peptide and endogenous insulin are secreted in equimolar amounts. C-peptide is secreted at a constant rate, having half-life more than that of insulin and is not cleared in the liver unlike insulin which undergoes first pass hepatic metabolism [7]. Owing to these advantages it is used as a testing method to determine the amount of insulin in the body and function of pancreatic beta cells.

3. MARKETED INSULIN:

Both short acting or rapid acting and intermediate acting types of human insulin are available. Porcine and Bovine insulin have been withdrawn from market.

1. Regular Insulin- It is a short acting, clear solution at neutral pH. After, subcutaneous administration it takes 15- 30 min to show its therapeutic response and it lasts for 6 to 8 hours.

2. NPH or Isophane Insulin- Protamine, a positively charged protein is added to regular insulin to prolong its time of action. Such type of insulin is called as NPH and its action lasts for 12 to 15 hours as it is absorbed very slowly from the subcutaneous tissue. Most commonly used at bedtime due to its extended half-life.

3. Lente Insulin- Here, zinc is added in excessive amounts to form an insoluble insulin-zinc complex and according to amount of zinc added it is classifying as Semelente, Lente and Ultralente [3].

Table 1. Marketed Insulin [8]

Generic name	Brand name	Manufacturer
Rapid Acting: duration of action up to		
Insulin aspart	NovoLOG	Novo Nordisk
Insulin lispro	HumaLOG	Eli Lilly
Insulin glulisine	APIDRA	Sanofi
Short acting: duration of action upto 8 hrs		
Insulin regular	HumuLIN R2	Eli Lilly
Insulin regular	NovoLIN R2	Novo Nordisk
Intermediate acting: duration of action upto 12 hrs		

NPH	HumuLIN N	Eli Lilly
NPH	NovoLIN N	Novo Nordisk
Long acting: duration of action upto 24 hrs		
Insulin detemir	LEVEMIR	Novo Nordisk
Insulin glargine	LANTUS	Sanofi
Insulin glargine	Basaglar	Lilly
Ultra long acting: duration of action upto 42 hrs		
Insulin degludac	TRESIBA	Novo Nordisk
Insulin degludec+liraglutide	Xultophy	Novo Nordisk
Mixtures:		
75% insulin lispro protamine and 25% insulin lispro	HumaLOG Mix 75/25	Eli Lilly
50% insulin lispro protamine and 50% insulin lispro	HumaLOG Mix 50/50	Eli Lilly
70% NPH and 30% insulin regular	HumuLIN 70/30	Eli Lilly
70% NPH and 30% insulin regular	NovoLIN 70/30	Novo Nordisk
70% aspart protamine and 30% insulin aspart	NovoLOG Mix 70/30	Novo Nordisk

70% insulin degludec, 30% insulin aspart	RYZODEG 70/30	Novo Nordisk

Premixed formulations of regular and NPH insulin are also available in market. Non-invasive alternatives to injected insulin can increase patient compliance but countless technical challenges are involved [9]. Regular Insulin has a slow onset of action because it has to come into the monomer form from the hexamer form in which it is administered before showing a response. Insulin analogs are used now a days which have modified protein sequences and hence stay in monomeric form itself.

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Sites for Insulin injections are subcutaneous tissues of thigh, upper arm, abdomen and buttocks. Rotation of site of application is very important to prevent side effects. Due to repeated infusions in same area of body fat lumps are formed under the skin (Lipo-hypertrophy) or there is loss of localized fat tissues (Lipoatrophy) [3]

4. INSULIN ANALOGS:

Insulin therapy for diabetic patients requires frequent blood samplings and numerous insulin injections which eventually leads to hypo-glycemia. Fluctuations of blood glucose due to hypo-glycemia especially at night presents a challenge before us to find such analogs which have a good glucose control.

4.1 Long-Acting Analogs:

4.1.1 Detemir:

Introduction:

A basal insulin analog – Detemir (Levemir), soluble at neutral pH, binds to albumin reversibly and has different molecular structure from that of human insulin. The change occurs due to the deletion of B30-threonine and the addition of a C14 fatty acid chain, myristic acid to B29-lysine. This leads to delayed dissociation and absorption [10].

Pharmacodynamic properties:

Insulin Detemir after injected forms a depot in subcutaneous tissue as hexamers and binds to both free and tissue bound albumin. Detemir hexamers, dimers, monomers all bind to albumin, but the dimers and hexamers penetrate very slowly than monomers [11]. The 2 main causes behind variability in action are ruled out in case of detemir namely the poor solubility (no requirement of resuspension as it is soluble) and no precipitant formation. It has a dose-dependent duration of action but as it is very predictable once or twice a day dose can be prescribed accordingly [10].

Pharmacokinetic properties:

The absolute bioavailability of detemir is approximately 60%. It is administered subcutaneously and it takes 6-8 hours to reach maximum concentration. The high binding of insulin detemir to albumin leads to slow distribution to peripheral tissues. Insulin detemir does not show variable absorption, the reason being this albumin binding and the blood flow at target sites has little or no effect on detemir absorption. Insulin detemir has a volume of distribution of approximately 0.1L/kg and it does not generally cross the blood brain barrier (BBB). All metabolites are inactive and in type 1 diabetes patients, an elimination half-life of 5-7 hours is apparent [12].

Dosage:

A 0.4 U/kg dose generally gives a 20-hour long duration of action and hence gives you a chance to give a once daily dose. But for doses lower than this you may need to administer twice daily. Insulin detemir's dose dependent duration of action allows for an easy predictability and conveniently once or twice daily doses can be chosen [10].

Therapeutic Efficacy:

A randomized trial on T1DM patients for six months was studied. They were given Insulin detemir or NPH with aspart and comparison of results led us to the conclusions that glycemic control was similar in both the groups, detemir reduced the risk of nocturnal hypoglycemia, more smoother plasma profile curves at night and also body weight of patients treated with detemir was considerably lower than that of with NPH. Another trial studied compared once-daily insulin detemir with NPH in combination with human insulin. The FBG readings were analyzed, which showed that insulin detemir showed less variability during the course of treatment than NPH. The hypoglycemic risk in detemir was lower by 26% than in NPH10.

4.1.2 Glargine:

Insulin glargine is a recombinant DNA-produced counterpart of human insulin which is long-lasting in nature and is made to fulfill the basal insulin needs of type 1 and 2 diabetes. When the human insulin molecule is modified at position A21 and the C-terminus of the B-chain, a stable product is formed that is soluble at pH 4.0. However, it forms amorphous microprecipitates in subcutaneous tissue from which modest amounts of insulin glargine are progressively released. In comparison to normal human insulins, the plasma concentration versus time profile of insulin glargine is generally consistent, with no obvious peak throughout 24 hours. As a result, once-daily delivery as a basal therapy is possible 13–15.

Pharmacokinetic Properties-

After the administration of subcutaneous injection of glargine in patients with diabetes and healthy subjects, the absorption of insulin has shown a more prolonged profile of serum concentration. Compared with NPH insulin, the concentration profile is constant over 24 hours, having no prominent peak [16]. Glargine's absorption rate appears to give a consistent basal insulin level for a day at least. In healthy participants, in comparison to NPH insulin, the absorption of glargine with 15 or 80 µg/mL zinc was substantially slower after administration as a subcutaneous injection. Importantly, regardless of the site of administration of glargine (leg, abdomen or leg) as a standard formulation having zinc 30 µg/ mL in healthy volunteers, absorption of the drug was similar [17].

Pharmacodynamic Properties –

Glargine is made utilizing recombinant DNA technology and a nonpathogenic Escherichia coli strain, with two structural changes to natural human insulin. At the NH2 terminal end of the B-chain, the addition of two arginine molecules is done, which changes the isoelectric point to 6.7 pH [18]. Also, due to these changes, when injected in the subcutaneous tissue at neutral pH, it precipitates and results in delayed absorption, with a prolonged duration of action than other exogenous basal insulins. Changes to the C-terminal amino acids do not affect insulin function because they are not involved in receptor binding [19]. For crystallization in the subcutaneous tissue, the inclusion of a slight amount of zinc (30 mg/L) to glargine is required which prolongs the absorption period even further [20].In a trial of 20 patients having type 1 diabetes, with the exception of the aforementioned study design flaws, the activity of a subcutaneous glargine injection qualitatively replicates the action profile of an equal dose of lispro insulin injected over 24 hours as CSII, with substantial intersubjective pharmacodynamic variability. As a result, this research shows that a subcutaneous injection of the long-acting insulin analog glargine can be used to replace basal insulin in type 1 diabetes [21].

Dosage

It is a once-a-day subcutaneously self-administered insulin to maintain basal insulin levels. It is approved for use in adults with, as well as children aged 6 and above with type 1 diabetes in the United States [15].

Therapeutic efficacy

In a trial of 4 weeks with 256 patients having type 1 diabetes, patients who recieved glargine had considerably lower levels of fasting plasma glucose (FPG) than the patients with NPH insulin. In patients who had received prior twice-daily NPH insulin, glargine was found to be better than NPH insulin in lowering FPG levels, although not in patients who'd already been administered with once-daily NPH insulin. Patients who used glargine had more steady fasting plasma glucose levels than those who used NPH insulin. In the study, glargine was confirmed as more effective and better than NPH in decreasing FPG levels [22]. In another study of 534 patients, glargine had lower FPG levels and fewer incidents of hypoglycemia in patients with type 1 diabetic when compared to once- or twice-daily NPH insulin [23].

4.2 Rapid Acting Insulin Analog:

4.2.1 Insulin Aspart:

Insulin Aspart is a rapid acting insulin analog having similar trends to that of human insulin got approval in the year 1999 from USFDA [24], structurally it replaced proline group from insulin B chain with aspartic acid at 28th position [25] and it shows minimal amount of side effects when administered but due to rapid acting property it is used only for specific purpose.

There are many factors that can bring about a change in the dosing system, mainly the route of administration, diet, physical workout, etc. Generally, 0.4-1 unit/kg of TDD may be given daily to adults for children greater than 2 are given the same dose as adults.

Since it has a rapid onset of action the peak is reached within 45-80 minutes of action; thus, it is generally advisable to give it with a long-acting insulin for a longer action and should be taken just before the food [25], giving subcutaneously also ensured some slow release but the half-life remains in minutes, the difficulty of mixing the two insulin (rapid and long acting) due to different stable pH was also overcome

Pharmacodynamics:

Human insulin and Insulin Aspart share almost the same lines in terms of potency and bioavailability but having the difference in onset of action. Insulin Aspart is generally given through a subcutaneous route; according to a study on [20] healthy volunteers received doses of Insulin Aspart or human Insulin, it was found that Insulin Aspart had a greater absorption having a greater GIRmax than human insulin [26, 25].

4.2.2 Insulin Lispro:

Introduction:

Humalog or **Insulin lispro** is a type of recombinant, rapid-acting human insulin analog. The difference from the original human insulin lies in the transposition of the 2 amino acids. It is equally potent to regular human insulin but with a smaller duration of hypoglycemic action as well as a quick onset of action. As a result of this, it imitates the physiological response of human insulin to ingestion of food to a greater extent.

Pharmacodynamics:

Insulin lispro has an identical primary structure as in regular human insulin, apart from the transposition of 2 amino acids i.e., at 28th and 29th position on the B chain from proline-lysine to lysine-proline. This slight modification of the sequence of amino acids leads to a reduction in self-association (as compared to regular human insulin). As a consequence of that, quicker absorption and lesser duration of action of insulin lispro take place.

In the pharmaceutical preparations of this insulin analog, it forms, with phenol and zinc hexamers that are stable and dissociate rapidly into monomers when injected subcutaneously, whereas in regular human insulin, the dissociation takes place slowly.

Insulin lispro, in comparison with regular human insulin, is slightly less to equally potent in binding to HIR (Human Insulin Receptor) and slightly more potent in binding to IGF-I (insulin-like growth factor type 1) receptor. Also on a molar basis, insulin lispro is equipotent when compared with regular human insulin in terms of glucose-lowering activity. However, it has a quicker onset of hypoglycemic activity i.e. it begins within 15 minutes as well as peaks prior and has a lesser duration of action as compared to regular human insulin. This implies insulin lispro has rapid absorption and elimination rate in the human body.

Therapeutic efficacy:

Type 1 diabetes patients that received insulin, intensive before meal administration of insulin lispro decreased average 2-hour postprandial glucose levels in a similar fashion as happens in regular human insulin regimen, whereas pre-prandial or fasting level of blood glucose were very much alike for both the insulins. As a whole, HbA1c values i.e., long-term glycemic control was more or less comparable for both the insulins. Hypoglycemia risk was is lesser with insulin lispro as compared to regular human insulin as found in few of the trials.

Dosage:

Administration of insulin lispro subcutaneously is done shortly or within 15 mins before or immediately after a meal. For treating diabetic ketoacidosis or during surgery or in acute illnesses, insulin lispro can be administered intravenously. Though daily requirements of insulin are in the range of 0.5-1 unit/ kg / 24hrs, the dosages of this insulin analog are decided based on target glucose levels in blood, apposite for the patient. Thorough monitoring of glucose levels in blood is crucial to warrant adherence to required levels. Basal insulin is usually administered with insulin lispro in the insulin treatment in T1DM [27].

4.2.3 Insulin Glulisine

Introduction:

Insulin glulisine differs from human regular insulin (in terms of molecular structure) in the substitution of 2 amino acids, i.e., of asparagine with lysine at B3 and of lysine with glutamic acid at B29 of the B chain. Unlike insulin aspart and insulin lispro, insulin glulisine formulation comprises of polysorbate [20] in place of zinc. Problems like catheter obstructions and clotting are recurrent with insulin glulisine, which makes insulin aspart and insulin lispro the favored analogs of insulin for use in subcutaneous pumps.

Pharmacodynamics:

Insulin glulisine had a little quicker onset of action than the other insulin analog. The reason behind this could be the absence of zinc, as after injection, zinc might interrupt and delay the action and absorption of other rapid acting insulin analog by retarding the dissociation into monomers. On the other hand, insulin glulisine speedily dissociates after subcutaneous injection and is quickly absorbed from the tissues. This analog displays decreased dimer and hexamer formation, and favors formation of monomers, enabling faster absorption. This type of absorption profile outcomes in insulin glulisine's pharmacokinetic profile that thoroughly mimics the typical after meal response of insulin.

Therapeutic efficacy:

Type 1 diabetes patients that received insulin glulisine was noninferior to regular human insulin in case of adult patients and insulin lispro in case of both paediatric and adult patients. The change in HbA1c (glycosylated hemoglobin) levels from baseline to the end of the study period, was specified as the primary endpoint in most of the clinical trials.

Dosage:

Insulin glulisine is to be administered at a dose based on the individual's blood glucose level. As stated before, daily insulin requirement falls in the range of 0.5 U/kg/day to 1.0 U/kg/day. Insulin glulisine can be administered, subcutaneously, 15 minutes before taking a meal or immediately after taking a meal. It's intended to be utilized in a regime that consists of an intermediate/ long-acting insulin. As in circumstances of other insulin analog, insulin glulisine dosages may entail modification and monitoring in patients that take concomitant drugs that can intensify glucose-lowering effects and in so doing upsurge the risk of hypoglycemia or that diminish the glucose-lowering effect [28 29, 30].

5. CURRENT INNOVATIONS:

5.1 Pulmonary delivery of insulin:

As compared to rectal, nasal, buccal formulations of insulin, insulin delivery via the pulmonary route has shown increased bioavailability. The remarkably large surface area of the highly vascularized lungs and huge capillary network of alveoli allows for quick onset of action. Due to the absence of enzymes of GIT like peptidases that break down insulin before it can act, this route of delivery is considered advantageous.

Inhaled insulin delivery formulations can be classified into 2 main categories: liquid and dry powder. Up until now, several developments have been done in these systems, namely Exubera, AIRs Insulin, AERxs iDMS (Insulin Diabetes Management System), TI (Technosphere insulin). FDA approved Exubera in 2006 but in 2007, Exubera was pulled from the market as a result of poor acceptance and poor sales.

Afrezza is a combination of drug + device that constitutes TI, pre-metered into one-time--use dose cartridges.

TI (Technospheres Insulin), an inhalation powder is composed of recombinant human insulin that is absorbed by Technosphere particles with fumaryl diketopiperazine powder (FDKP) as the excipient carrier. FDKP forms microspheres by self-assembly in a moderately acidic environment through hydrogen bonding. In the time of precipitation process, molecules like proteins and peptides (insulin in our case) can be introduced and a powder is formed of the particles that are freeze-dried. FDKP is highly water-soluble, whereas the particles are promptly dissolved in pH that is either neutral or basic. During the process of inhalation, the dissolved particles (in the neutral pH environment of the lung) are absorbed quickly into the systemic circulation. Of the dose inhaled, 59% reached the lung and the rest of the dose, i.e. 10% is in the stomach and 30% in the oropharynx.[31]

The issue with this inhaled human insulin formulation is that the HbA1c reduction is lower as compared to subcutaneous insulin. Optimizing this inhaled insulin formulation is required for it to become an alternative that's equivalent to subcutaneous insulin.[32]

5.2 Liver Preferred Insulin / Hepatoselective Analog:

In the case of conventional insulin therapy of subcutaneous administration, insulin is delivered to the liver via the portal vein, where half of the insulin is hepatically absorbed. This leads to high peripheral tissue concentration compared to hepatic exposure, causing an escalated risk of weight gain, hypoglycemia and insulin resistance. This constraint can be controlled by the usage of hepatoselective insulin analog wherein insulin therapy safety is more increased by restoring the physiological balance between the hepatic and peripheral actions of insulin. [32 33 34]

In the cases of insulin PEGLispro, insulin detemir, thyroxyl insulin conjugates, and proinsulin, hepatoselectivity has been achieved to numerous extents. With insulin detemir and thyroxyl conjugates, hepatoselectivity has been demonstrated through molecular size increment by binding to endogenous proteins. With insulin PEGLispro and proinsulin, hepatoselectivity has been denoted through molecular size increment via their design. [33 35]

Briefly, PEGLispro consists of insulin lispro bound to polyethylene glucose polymer (which is hydrophilic). Its increased molecular structure as the portal veins are more easily penetrated than the peripheral

capillaries.Its Phase 3 clinical studies show decreased weight gain, A1c, nocturnal hypoglycemia, and glucose variability. Nonetheless, this drug was discontinued due to its association with lipid abnormalities and liver enzymes. Other upcoming insulins are currently being designed to rearrange PegLispro that ensure hepatoselectivity. [32 34]

A recent investigation of a ‘’proinsulin–transferrin fusion protein’’ was conducted in mice with diabetes for its preferential activity that displayed a absence of peripheral activity in an immunoprecipitation assay.[33 35]

It was predicted that PEG can have a toxic effect on the liver or hepatoselective insulin analog can lead to increased liver steatosis, caused by exposure of the liver to insulin in situations of decreased suppression of lipolysis by insulin in the peripheral adipose tissues, resulting in free fatty acid fluxes towards the liver. Hence even though they are appealing, hepatoselective insulin analog persist as tricky. [32]

5.3 Smart Insulin Analog

The goal of lessening the number of insulin injections along with reducing hypoglycemia has created a sense of the development of ‘’glucose-sensitive insulin’’ or ‘’smart insulin’’ that are effective and safe and thus, can improvise insulin therapy to a whole another level. [36]

‘’Smart insulin’’ include formulations in which molecular entities are incorporated within the insulin molecule or with fusion proteins or linked carriers. The entities could be gels, polymers or lectins. These formulations are engineered in such a way that when triggered by higher glucose levels, they will liberate insulin or switch insulin to a bioactive conformation in proportion to hyperglycaemia gradients. [33 36]

Also known as ‘’Glucose Responsive Insulin (GRI)’’, they can be classified by their method of insulin release or glucose detection as follows: [33]

1. Mechanical GRI systems that are algorithm based
2. Polymer-based systems: In this, insulin is enclosed inside a vesicle or hydrogel made of a glucose-responsive polymeric matrix. The sensing of glucose is attained by one of the following techniques:
    a) The method involving detection of glucose based on its affinity to ConA (concanavalin A, a lectin/ carbohydrate-binding protein)
    b) The method involving the formation of gluconic acid via glucose oxidase catalyzes the enzymatic reaction of glucose (GOx).
    c) The method based on PBA (phenylboronic acid) and glucose interaction. [33]
3. Molecular glucose-responsive insulin analog systems, which includes the addition of a glucose-responsive motif to the insulin molecule or its formulation, which imparts glucose-responsive alterations in bioavailability or hormonal activity in either scenario [37]

INTRINSIC/ UNIMOLECULAR GRIs: In these types of analogs, the modified hormone offers glucose-dependent activity or bioavailability to the modified hormone.

A few of the examples of these systems include:

→ **Insulin fusion proteins**: This is insulin–glucose oxidase fusion molecule

→ **Biology-inspired GRI systems**: An appealing frontier of GRI engineering makes use of an endogenous biological system that has been 'hijacked' in its natural state to offer an active component.

→ **PBA-modified insulin derivatives** PBA is a carbohydrate-sensing diol-binding element. PBA may be attached to the B29 position of insulin without compromising its biological action, allowing it to bind diol-containing sequestering agents. [37]

5.4 Oral insulin delivery

Insulin is administered via the subcutaneous route for treating diabetes. This is so because the high hydrophilicity and molecular weight of proteins obstruct/inhibit insulin’s intestinal absorption, causing increased variability, less oral bioavailability, and insignificant plasma levels.

To prevent using needles, risk of contamination, immune reactions, pain and improve upon patient compliance, the oral route of administration can be considered better. Moreover, with the oral route, there's higher patient acceptance and cost-effectiveness associated with it.

Once administered orally, insulin tends to mimic the typical pathway of insulin in the body after endogenous secretion, resulting in improved glucose homeostasis. This route also ensures increased portal vein concentrations, with no prolonged peripheral hyperinsulinemia, which is links with retinopathy and neuropathy.

A few of the limitations with this route include poor bioavailability caused by the degradation of insulin in GIT via severe pH physiological conditions, proteolytic enzymes and low permeability throughout the intestinal epithelium.

Nevertheless, the oral delivery route is still considered as an alternative one for the administration of insulin and is being developed more to overcome its limitations and meet the level of safety and efficacy newer insulin analog injections have reached so far. [36 38]

Some of the oral insulin formulations developed so far are as follows. Some of these are marketed formulations whereas some are in the clinical trials phase.

Table 2. Oral Insulin [38]

NAME OF THE PRODUCT	**TECHNOLOGY**
Capsulin	An enteric-coated capsule containing a mixture of insulin, a solubilizer, and an absorption enhancer
ORMD-0801	Enteric-coated capsule filled with insulin and excipients to promote the insulin's intestinal uptake and protect it, co-formulated with some enzyme inhibitors
ORA2	Capsule constituting insulin in a matrix of dextran
-	Eligen®; capsule filled with insulin along with an absorption enhancer, facilitating the passive transport (transcellularly)
NN1952	tablet/capsule consisting of absorption enhancers which are known to activate formation of micelle, facilitating insulin transport
NN1953; NN1954	Tablet made of long-acting insulin analog
IN-105	Insulin altered with a small Poly (ethylene glycol) (PEG)
HDV-I	Liposomal insulin, also known as HDV-I (hepatic directed vesicles–insulin), an orally delivered type of hepatic directed vesicles–insulin.
-	Insulin-loaded bioadhesive nanoparticles from NOD Technology
-	Polymer or nanoparticle containing insulin, covered with vitamin B12 for the purpose of targeted delivery

5.5 Once weekly insulin

Insulin icodec, one of the insulin analogs designed for oral route of administration, and of which OI338 displayed positive results in clinical studies. The molecular alterations done with insulin icodec induced properties that made it apt for ‘’once in a week’’ dosing and thus can be categorized as a basal insulin.

The backbone of icodec includes 2 substitutions (A14Glu and B25His); first done in OI338, that improved proteolytic stability of insulin.

Another substitution of the native Tyr at B16 to His is known to significantly decrease receptor affinity and stronger albumin binding. This in turn reduces the time taken by insulin molecules to undergo the process of receptor mediated clearance. This leads to an inactive albumin-bound depot forming that provides slow and continuous initiation of insulin action as well as extending its action throughout a week. This way of lowering basal glucose over a full week with administration of insulin icodec’s once weekly dosing grants additional benefit of lesser injections. [39 40]

For maintaining the required efficacy and safety profile in patients with T1DM, considerable research and studies of these once-weekly insulin analog are being investigated. [36]

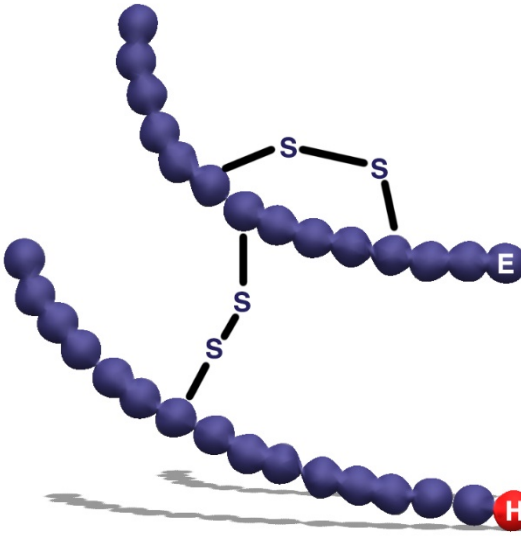

Fig. 1. Sequence of insulin icodec [40]

5.6 Ultrastable insulin:

In the developing countries which have limited access to refrigeration, the complicated and expensive cold-chain logistics related to insulin analogs are an issue when it comes to diabetes therapy. When the required conditions of the cold chain aren’t maintained, it results in rapid protein conformational modifications in insulin molecules. These in turn lead to amyloid fibrils formation.

Fibrillation is an irreversible method that can be a grave challenge to insulin efficacy, safety and storage.

To conquer this kind of situation, research of ultrastable insulins with improved thermal stability is being conducted. SCI-57, i.e. single-chain insulin analog with 57 residues is a potential candidate being investigated currently that can resist this problem of thermal fibrillation in vitro as well as display biological properties equivalent to original insulin animal models (in vivo) [33 34 41].

5.7 Antibody-linked insulins:

Though the research on this topic is still undergoing and is in animal model phase, these insulin analogs might be used to attain weekly administration of insulin formulations, These have a protracted action profile caused due to reduced clearance. [28]

5.8 Co-therapy with incretin hormones:

A combination of GLP1 (Glucagon-like peptide-1) analog and insulin degludec, IDegLira is currently being investigated to treat diabetes. A notable feature about this analog is that it decreases insulin dosing, along with reduced weight gain and lower frequency of hypoglycemia. These do have unfavorable effects like nausea, flatulence, and gastrointestinal issues. Nevertheless, the promising results obtained from the later stages of clinical trials are of extreme

importance, especially in cases of diabetes linked with too much weight gain. [33]

Few of the other developments of insulin analog like receptor isoform selective analog [33] and Co-formulations [28] are still under investigation and thorough studies could let us know more about their efficacy and safety in T1DM management.

6. INSULIN ANALOG AND CANCER

With the progressive development and need for alternative sources, analogs of insulin came into the market, but with orange there is always a bitter seed, Cancer being one of the disease having a wide spread looks forward to further increase its rising territory by connecting with Insulin analogs but there is still not any conclusive report and the study on the same is still in process [42].

"Having an increased amount of cake is good for taste buds but not for health", same is the case with insulin analogs. Analogs which cause increased mitosis than normal human insulin (because of change in the structure than human insulin), simulation of IGF-1 receptor are often a cause for cancer [24]. Further if the analogs have greater affinity towards IR IR-A and IR-B) and IGF-1R it can lead to cancerous effect [43], If the Tumor is left undiagnosed and insulin analog is given which can cause increased mitogenic effect may activate the cells because of increased IR expression and can worsen the disease [24].

Concentration of drug also have a significant effect, some amount of insulin analogs requires higher concentration to reach the target metabolic activity and thus chances of causing cancer increases(dose-dependent) [43]. According to a study it was found that analogs exposure have a cancerous effect on stomach, breast, kidney and a reduced/no risk on prostate cancer [44].

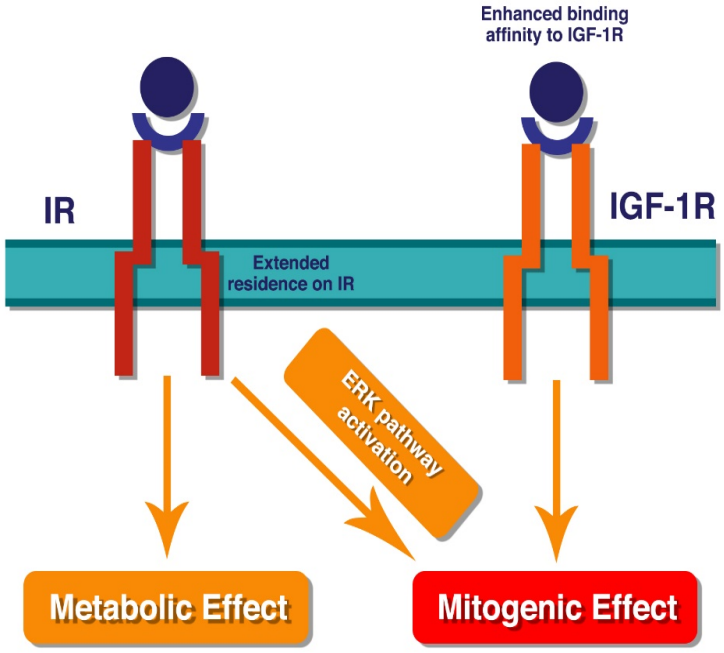

Fig 2. Molecular mechanisms potentially involved in the mitogenic effects of long-acting insulin analog [24].

Glargine being one of the long-acting insulin was questioned many times based on its safety profile since it had a greater affinity towards IGF-1R, thus continuous use of Glargine for a longer time has a side effect and may cause breast cancer; it is still used in market since it has lesser side effects of cancer. AspB10 insulin analog was discontinued during its developmental stage because it showed an increase in both benign and malignant tumor, thus can be considered carcinogenic in nature [45]. Insulin analog Lipro is generally used since it has a very less/NO carcinogenic effect and having onset of action of 15-30 minutes [46]. Most of the rapid-acting insulin have non-carcinogenic effect and thus is widely used [47].

Cancerous effect due to Insulin analogs is a complex study and has been highly debatable [48], many studies does not point to a conclusive statement since two groups have different perspective on the same, but having a long exposure of the analogs may have certain side-effect. The available data have pointed

out for it to be safe but there is a say “World is full of unexpectation” [43].

7. CONCLUSION

Interestingly one can observe that insulin is still being investigated for over a century since its discovery demonstrates its medicinal significance and complexity. Insulin action of current innovations responsive to variations in blood glucose concentration remains a fundamental target, which should then be combined with additional pharmacology that tackles the underlying molecular pathology inherent to human diabetes' heterogeneity. Some of the reviewed analogs are Detemir, Glulisine, Aspart, Glargine, Lispro which include both rapid-acting and long-acting analogs. Their pharmacodynamics and therapeutic profile is superior to basal insulin. These therapy approaches do, however, have negative effects which have opened doors for further innovations. Few of these include pulmonary delivery of insulin, liver preferred insulin, oral insulin delivery, once weekly insulin (insulin icodec), ultrastable insulin, antibody-linked insulins, receptor isoform-selective analogs, and co-formulations. Apart from these, co-therapy with the incretin hormones type of analog’s most prominent property is that it reduces insulin dose, as well as weight gain and the frequency of hypoglycemia. Smart insulins are mechanically engineered to control glycemic control according to the need. It's easier to visualize that by the next century, the traditional insulin will be obsolete and it will be substituted by newer technology.

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