ВОЛГОГРАДСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ

Biotechnological pharmaceuticals. Insulins.

Biotechnological medicinal products (BtMPs)

Biotechnological medicinal products ('BtMPs') drugs manufactured using biotechnological processes and methods (including DNA recombinant technology, technology for controlled expression of genes encoding biologically active proteins in prokaryotes and eukaryotes, including modified mammalian cells), hybridoma method and monoclonal antibody method.

There are several historical stages in the development of biotechnology:

- Historical times - production of foods and beverages based on microbiological fermentation.
- Since the end of the 19th century - production of chemicals (citrate, acetate) and solvents (acetone, ethanol, butanol, isopropanol).
- Since 1940 - synthesis of antibiotics.
- Since 1960 - production of proteins and peptides.
- Since 1980 - synthesis of steroid hormones.
- Since 2000 - industrial production of monoclonal antibodies.

Prospective directions of biotechnology development:

- genetic engineering (hormones for humans, vaccines, transgenic plants and animals);
- production of primary and secondary metabolites (amino acids, vitamins, interferons, vaccines, antibiotics, etc.);
- enzymology engineering (enzymes, biosensors and biochips);
- cell and tissue engineering of plants (callus and suspension cultures of plants);
- environmental biotechnology (utilisation of solid, liquid and gaseous waste).

Differences between biotechnological processes and the production of synthetic drugs

Compared to chemical technology, biotechnology has a number of the following main advantages:

1.The possibility of obtaining specific and unique natural substances, some of which (e.g. proteins, DNA) are difficult to obtain by chemical synthesis.

2.Carrying out biotechnological processes at relatively low temperatures and pressures.

3.Microorganisms have significantly higher growth rates and cell mass accumulation than other organisms. For example, using microorganisms in a 300 m3 fermenter, 1 tonne of protein (365 tonnes/year) can be produced in a day. To produce the same amount of protein per year using cattle, you would need a herd of 30,000 cattle. If leguminous plants, such as peas, were to be used to produce protein at this rate, it would require a field of peas covering 5,400 hectares.

4.Biotechnology processes can use cheap agricultural and industrial wastes as raw materials.

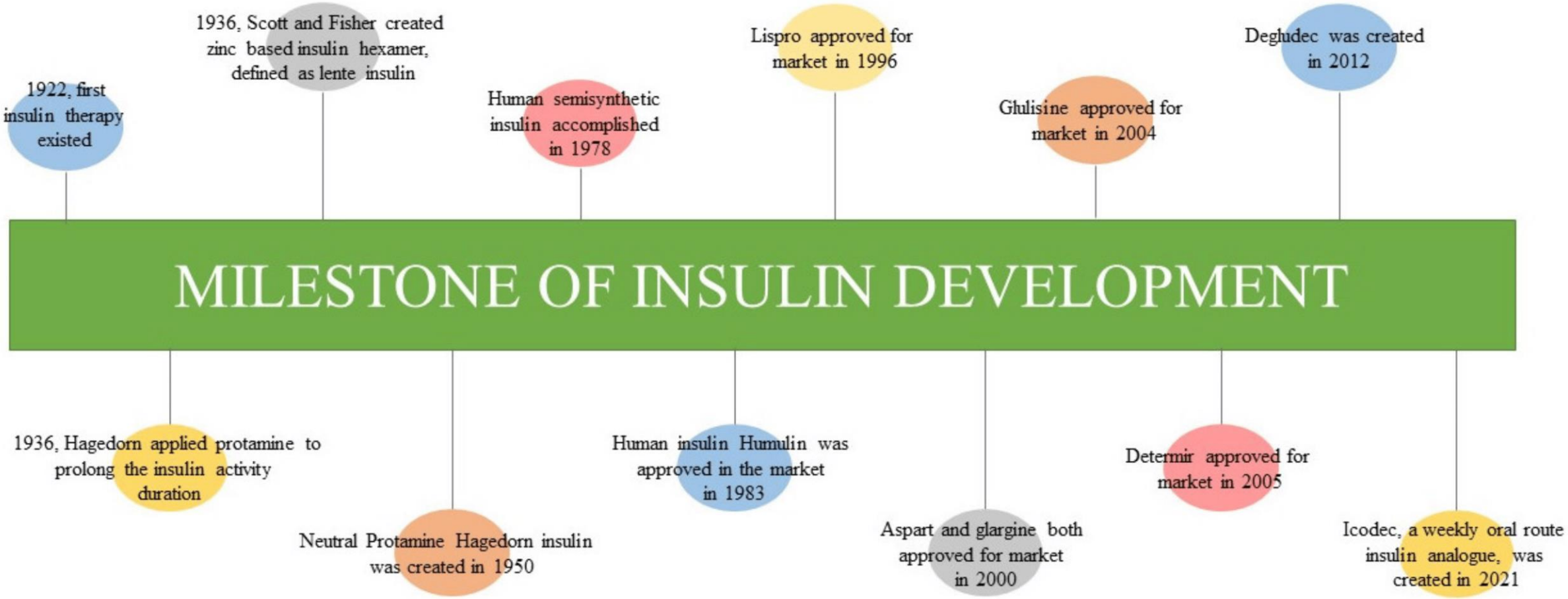
5.Compared to chemical processes, biotechnological processes are usually more environmentally friendly, have less harmful waste, and are close to natural processes occurring in nature.

6.As a rule, technology and apparatus in biotechnological productions are simpler and cheaper. The USA and Japan, which have accumulated many years of experience in biotechnology for agriculture, pharmaceutical, food and chemical industries, are considered to be the leaders of biotechnology today. The countries of Western Europe (Germany, France, Great Britain, Switzerland), China, India and Russia have a strong position in the production of enzyme preparations, amino acids, proteins and medicines. These countries are characterised by a strong potential of engineering and technology, intensive fundamental and applied research in various areas of biotechnology.

Groups of drugs produced by biotechnological methods.

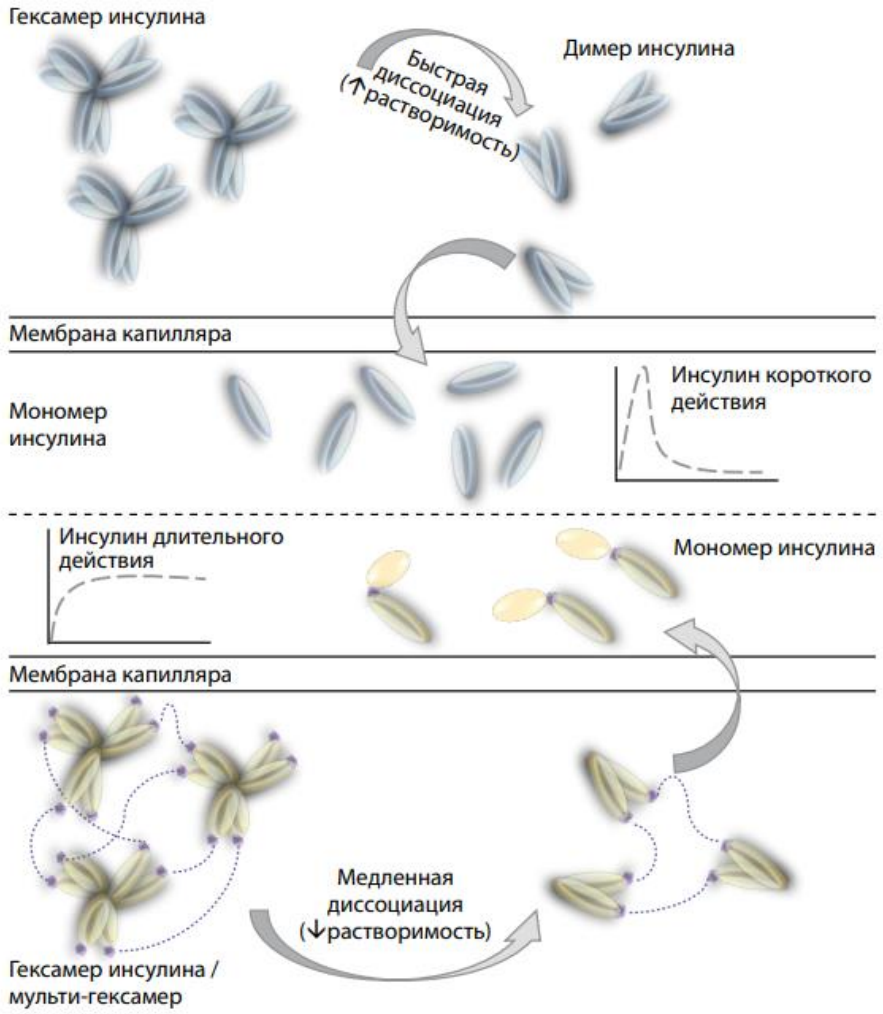
- antibiotics (penicillins, macrolides, tetracyclines, streptomycin);
- hormones (insulin, steroid hormones, somatotropin);
- monoclonal antibodies;
- cytokines (interferons);
- new generation vaccines;
- probiotics (lactobacterin, bifidumbacterin);
- serums (against snake and insect venom);
- callus cultures of plants (suspension culture of ginseng cells);
- enzymes (streptokinase, amylase, lipase, protease) and their blockers;
- vitamins (B2, B12, D3);
- amino acids (lysine, tryptophan);
- dextrans (plasma replacement solutions);
- alcohols (ethanol); pollen allergens;
- low molecular weight heparins;
- stem cells.

Evolution of insulin therapy



Human insulins

Insulin exists as monomers and dimers with a natural tendency to aggregate into hexamers, the exact position of three disulfide bonds in the structure of proinsulin (and the position of two bonds in processed insulin) was determined, the N- and C-terminal regions of the A chain and the hydrophobic residues of the B chain were established in aggregate, this allowed not only a better understanding of physiology and pharmacology, but also brought the dawn of genetic engineering and the synthesis of human insulin much closer.



Human insulins

Recombinant human insulin was completely homologous to the human hormone, had a low risk of immune response, and was much cheaper to produce.To reproduce physiological prandial insulin secretion, ideal insulin must be rapidly absorbed into the bloodstream from the subcutaneous fat.The tools for insulin administration have also undergone significant changes: from large, reusable glass syringes and needles that had to be sterilised before use, to disposable plastic syringes, syringe pens, infusion systems and virtually painless injections.

MODERN INSULIN ANALOGUES

Insulin lispro

The first fast-acting insulin analogue, insulin lispro, was developed in 1996 by Richard Di Marchi and his research group at Lilly laboratories. The scientists noticed that insulin-like growth factor-1 is structurally similar to insulin, forming hexamers with lower bonding strengths, allowing it to dissociate more rapidly into monomers. They found that this is due to an amino acid sequence difference in the C-terminal positions 28 and 29 of the B-chain between IGF-1 (Lys-Pro) and insulin (Pro-Lys). ‘Simple’ inversion of the two amino acids provided faster dissociation of insulin hexamers after subcutaneous injection with faster absorption into the bloodstream and shorter duration of action. In clinical studies, lispro insulin has been shown to reduce postprandial glycaemia better than human insulin. Lizpro begins to take effect approximately 15 minutes after subcutaneous injection and reaches maximum serum concentration (Cmax) in 30-60 minutes. The duration of action of insulin lispro is 3-4 h. The absolute bioavailability of the drug can reach 77% at doses from 0.1 to 0.2 U/kg. Lyspro insulin is equivalent to human insulin in terms of glycaemic lowering efficiency.

Insulin aspart

Aspart, a short-acting prandial insulin analogue approved and marketed in 2000, has a pharmacokinetic profile comparable to lispro, including its bioactivity, and is formed by replacing the B28 amino acid proline with an asparagic acid.This modification eliminates the monomer-monomer attraction surface and further enhances the repulsion between the charged asparagic acid and its neighbouring one. As a result, the insulin hexamer dissociates more rapidly to monomers and begins its action within 10-20 minutes after subcutaneous administration, reaching a maximum concentration in serum 40-50 minutes after the onset of action, which lasts for 3-5 h.Ultrafast insulin aspart, launched in 2017, contains L-arginine as a stabiliser and niacinamide to increase absorption by enhancing subcutaneous blood flow. Compared to conventional insulin aspart, this formulation results in an earlier (by 5 minutes) onset of action, rapid development of sugar-lowering effect (by 74% in the first half hour after injection) and a shorter duration of action by 14 minutes.

Insulin glulisine

Glulisine is another short-acting prandial insulin analogue compared to lispro and aspart; the drug was only introduced on the market in 2004. Glulisine is obtained by substituting two amino acids. Asparagine at position B3 is replaced by lysine and lysine at position B29 is replaced by glutamic acid. These changes increase the rate of transition of the hexamer into dimers and monomers after dissolution in the subcutaneous tissue, and also reduce the isoelectric point of insulin from 5.5 (native insulin) to 5.1, which significantly improves its solubility. Glulizin begins to act quickly (within 20 minutes after subcutaneous administration) and in 1 h reaches Cmax. The action of the drug glulisine lasts for 4 h, and its absolute bioavailability after subcutaneous administration is approximately 70%. In contrast to insulins lispro and aspart, insulin glulisin uses polysorbate 20 instead of Zn2+ as an excipient, which allows to achieve a faster onset of action. The removal of zinc leads to the breakdown of the insulin hexamer, which favours absorption.Translated with DeepL.com (free version)

Basal insulin analogues
Insulin glargine

In 2000, the first basal insulin analogue, glargine, was approved, which changed forever the long-held beliefs about the complexity and unsafety of insulin therapy. Glargine has two L-arginine residues added to the C-terminus of the B-chain and the replacement of glycine with asparagine. These modifications alter the isoelectric point of insulin and prevent the disamidating effect of asparagine, subsequently causing a more stable aggregation that prolongs release. As a result, deposition occurs only at neutral pH (at the injection site), whereas the molecule dissolves at acidic pH (in the vial). Insulin glargine has a more favourable and smooth pharmacodynamic profile than insulin NPH (Neutral Protamine Hagedorn (isophane-insulin, NPXH-insulin)), with a mean duration of action of 20 h after a single first dose, which increases with maintenance therapy. Switching from insulin NPH to insulin glargine results in improved overall glucose control, as reflected in lower fasting blood glucose levels and fewer nocturnal hypoglycaemias. Glargine begins to act 1-2 h after administration. The duration of action is about 24 h

Insulin detemir

Insulin detemir was the second analogue of long-acting insulin. In the insulin detemir molecule, amino acid B30 has been removed and the 14-carbon aliphatic fatty acid has been acylated to amino acid B29. This change ensures reversible binding between albumin and the added fatty acid, thus slowing down the absorption of detemir in peripheral tissues, since only the free analogue binds to the insulin receptor. The pharmacokinetic profile of insulin detemir is characterised by peak activity 6-8 h after administration and a duration of action of up to 24 h. Like glargine, detemir causes fewer episodes of hypoglycaemia compared to NPH. Given its relatively short duration of action, detemir is often administered twice daily to ensure sustained basal insulin coverage.

Insulin degludec

In 2012, a new ultra-long-acting insulin analogue, known as degludec, emerged that differs from the native molecule by the absence of the B-chain threonine at position 30 and the presence of a 16-carbon fatty bivalent acid (hexadecenoic acid) at the B-chain lysine at position 29 via glutamate acylation. These modifications allow the formation of multi-hexamers and depot complexes in the subcutaneous layer, which promotes slow release into the systemic bloodstream . Insulin degludec is available in concentrations of 100 and 200 U/ml, with a duration of action of up to 42 h (half-life 25 h). The prolonged action of this human insulin analogue allows more flexibility in the timing of administration, but limits/complicates its use due to the need for dose adjustment.

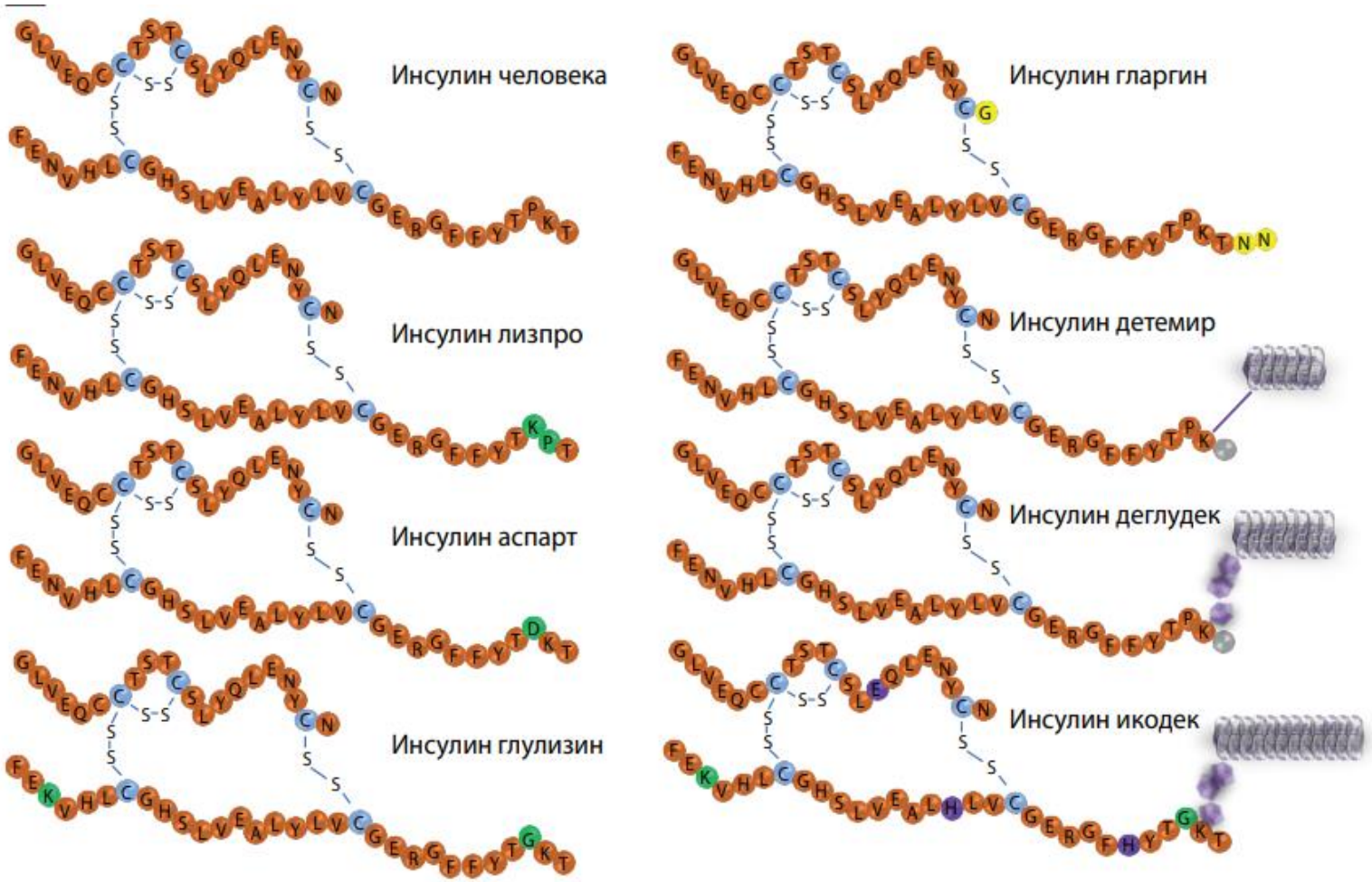
Insulin icodec (icodec) and BIF are weekly insulins

Icodeq is a relatively new insulin analogue administered once a week, with a plasma elimination half-life of 196 hours. Three amino acids have been replaced in the molecule of insulin icodec, and it contains a side-chain acylated C20-fatty bidentate acid. The molecular structure of the drug proved to be more stable than the native one with a longer half-life, lower receptor affinity, clearance and enzymatic degradation. Currently, icodec is undergoing phase 3 clinical trials. According to the results already published, icodec in basal-bolus therapy has shown comparable or slightly higher efficacy in reducing glycated haemoglobin (HbA1c) without evidence of increased risk of hypoglycaemia compared to insulin glargine in DM2 patients (both insulin-naive and those switched from insulin glargine 100 U/ml).Even longer-acting basal insulin analogues are currently being developed, including basal insulin Fc (BIF), whose extended half-life is attributed to immunoglobulin FcRn-mediated recycling, the same process responsible for maintaining serum IgG levels. Preliminary results have shown a prolonged sugar-lowering effect (up to 10 days after a single injection) comparable to that of conventional basal insulin, without increasing the risk of hypoglycaemia. Fig. 3 shows the structures of currently available insulin analogues and the mechanism of prolongation of their action.

Mixed insulins

In these preparations, a mixture of prandial insulin with medium-acting insulin in a single vial or syringe pen both provides post-meal glycaemic reduction and closes the need for basal insulin, as the pharmacokinetics of each individual component are preserved. Fixed-ratio premixed insulins can be used to improve adherence and convenience for patients with diabetes (DM1/DM2), as fewer injections are required compared with the traditional basal-bolus insulin therapy regimen. The pharmacokinetic and pharmacodynamic profiles of the prandial component in the mixtures remain unchanged, so they can be administered immediately before meals. Combinations of fast-acting insulin lispro and protamine-insulin lispro, and fast-acting insulin aspart and protaminaspart insulin in various ratios are available. It should be noted that the advantages in terms of glycaemic control and nocturnal hypoglycaemia are maintained with lispro and insulin-asparte mixtures compared with pre-mixed human insuli.na preparations, which do not have these advantages.

Structural analogues of human insulin



Fixed combinations of basal insulin and glucagon-like peptide type 1 receptor agonist

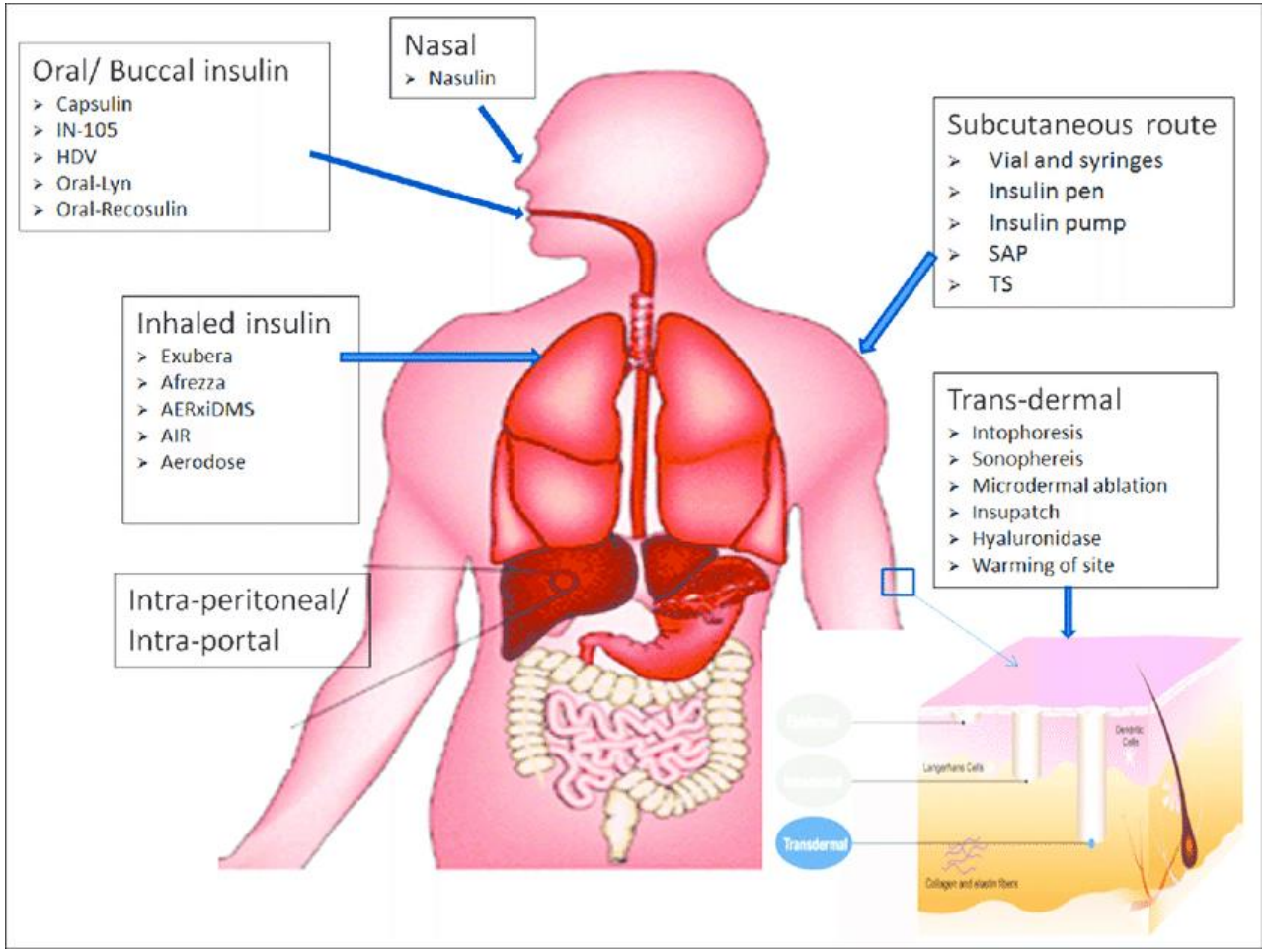
Currently, there are two fixed combinations of basal insulin with a type 1 glucagon-like peptide agonist: insulin glargine with lixisenatide (iGlarLixi) and insulin degludec with liraglutide (iDegLira). According to the extensive evidence base available, fixed combinations of glucagon-like peptide type 1 receptor agonist and basal insulin have greater sugar-lowering efficacy than the individual components, with less weight gain and lower incidence of hypoglycaemia than intensive insulin therapy regimens

Fixed combinations of pramlintide and insulin

Another interesting direction for improving the efficacy of insulin therapy is the development of fixed combinations of pramlintide with an insulin analogue. Pramlintide is an analogue of amylin. Similar to the endocrine hormone amylin, it has an antihyperglycaemic effect by reducing glucagon secretion, gastric emptying rate and food intake. In a recently completed Phase II comparative trial (NCT03981627) of a fixed combination of pramlintide with the fast-acting human insulin analogue A21G (a metabolite of glargine), patients required a lower dose of insulin before meals and reduced body weight (an average of 1.6 kg in 24 days), while patients receiving insulin aspart gained an average of 0.4 kg. Apparently, this is due to a decrease in appetite, which is one of the physiological effects of amylin and, consequently, pramlintide.

Novel routes of insulin for diabetes treatment

The injectable route of administration has been the most powerful barrier to the use of the drug since the discovery of insulin; for this reason, scientists began looking for alternative routes of administration soon after its introduction into clinical practice.



Inhalation route of administration

Advantages of the inhalation route:

- For example, the total surface area of the pulmonary alveoli is about 90 m2 , which provides greater opportunity for inhaled drugs to penetrate into the systemic bloodstream.

Disadvantages of the inhalation route:

- Concerns related to bioavailability, which was significantly less than that of subcutaneous administration, which in turn necessitates multiple administrations for optimal effect. 6 of the 4,740 patients taking Exubera during the clinical trial phase were found to have lung cancer.
- It should be mentioned, however, that all of these patients had previously smoked cigarettes, and the number of reported cases was too small to conclude a causal relationship between Exubera therapy and the development of lung cancer.
- A persistent side effect of inhaled insulin is coughing, so long-term studies of the safety and efficacy of the drug are needed.

Oral administration

Oral administration of insulin remains the most attractive prospect because of its convenience, safety and physiological entry of insulin into the portal circulation. Oral insulin delivery mimics the physiological (endogenous) pattern of insulin secretion by increasing portal insulin concentration after absorption in the intestine. In studies conducted, oral insulin also prevented autoimmune destruction of pancreatic β-cells. Oral administration of insulin is less costly as it does not require sterile conditions and is accompanied by a higher level of adherence due to reduced pain and discomfort from injections. However, the bioavailability of insulin is greatly complicated by physiological factors such as pH changes in the gastrointestinal tract (acidic pH in the stomach alternates with alkaline pH in the intestine), enzymatic breakdown and metabolism in the liver. Two main approaches have been developed to overcome these limitations. The first is protection against degradation in the acidic environment of the stomach, allowing the drug to be absorbed through the intestinal epithelium. Such a protective effect can be provided by micro-patches encapsulated in acid-resistant capsules. Once the capsule reaches the intestinal lumen, the coating dissolves, the particles are released and attach to the mucosa, after which insulin absorption occurs through the epithelium. This approach has been implemented in ORMD-0801, recombinant human oral insulin delivered by an oral polypeptide system containing excipients that promote absorption by inhibiting proteolysis in the small intestine and enhancing peptide translocation through the intestinal epithelial layer.

Insulin pump

Pump-assisted insulin administration restores the physiological porto-systemic insulin gradient, preventing chronic systemic hyperinsulinaemia. Recent work has presented the results of implanting intraperitoneal insulin pumps in animals and replenishing them with self-administered oral tablets. Tablets containing concentrated insulin, after ingestion, pass through a magnetic orientation system and attach to the intestinal wall where the insulin pump is located, allowing replenishment of the insulin reservoir by transmural puncture. The built-in battery of the device can be charged by external electromagnetic waves.

Intranasal delivery of insulin

Intranasal delivery has long been considered as a possible route of insulin administration. Including numerous studies have been devoted to investigating potential carriers and enhancers for intranasal delivery of insulin. Cell-penetrating peptides are categorised as being able to increase permeability across cell membranes. The effect of various cell penetrating peptides on nasal absorption of insulin has been evaluated in animal studies. It was observed that insulin administered together with the tested peptides (+L-R8, +D-R8, +D-penetratin and +L-penetratin) was absorbed significantly faster and in greater amounts. Specifically, the reduction in blood glucose levels after co-administration of +D-penetratin and +L-penetratin with insulin was 30 and 50%, respectively, compared to R8. PenetraMax and L-penetratin did not induce changes in the nasal mucosa. The study authors concluded that L-penetratin may be an effective enhancer of insulin penetration through the nasal mucosa, and these data suggest the potential use of the intranasal route for the development of insulin therapy

Transbuccal route of insulin delivery

This route has the same advantages as the nasal route in terms of low invasiveness. The oral cavity is essentially a mucosa with a large area and high vascularisation, which allows drugs to penetrate directly into the systemic bloodstream, thus ensuring a rapid onset of action. However, significant disadvantages include discomfort and irritation of the mucosa, which provokes swallowing of the drug. Despite this, buccal administration of drugs, especially proteins, is promising due to minimal invasiveness compared to multiple injections.

Transdermal delivery of insulin

The transdermal route of administration is also considered minimally invasive and could potentially be used for insulin delivery, as patches for percutaneous administration of other drugs have been used successfully for several decades. A wide range of different enhancers that facilitate insulin transport through the skin without significantly compromising insulin bioavailability and efficacy are currently under experimental investigation.

Vaginal and rectal routes of insulin delivery

Vaginal delivery of insulin is another potential route of drug delivery. It has a large mucosal area with an abundance of blood vessels that may help in the systemic delivery of therapeutic agents.

Therefore, insulin-chitosan (Ma/Su/GeB) encapsulated ascorbate nanoparticles were investigated, particularly to assess the ability of the lyophilised cylinder to release insulin-loaded nanoparticles into the vaginal mucosa. The in vivo insulin-chitosan gel with the addition of two other enhancing agents, taurocholate (TAU) and dimethyl-β-cyclodextrin (DM-βCD), was injected into the vagina and rectum of rats. The blood glucose concentration decreased to a greater extent in rats injected with DMβCD-chitosan gel both vaginally and rectally, confirming the feasibility of this route of insulin delivery to the body. Based on the success of controlled release of insulin, a study of Span 40 and Span 60 preparations was conducted on diabetic rats. 1.5 h after vaginal administration of insulin, a maximum reduction in blood glucose levels was observed with a sustained and prolonged hypoglycaemic effect.

Vaginal and rectal routes of insulin delivery

The adult rectum is 12-15 cm long, has an average surface area of 200-400 cm2 and a pH of 7.2 to 7.4. Rectal administration of insulin can be effective because portosystemic shunting and lymphatic drainage of the rectum play a significant role in the systemic absorption of lipophilic drugs.

- In this method of administration, the high rate and completeness of absorption of the drug into the systemic bloodstream are of great importance.
- Avoids the effect of the first passage of the drug through the liverthe administered drug is not degraded under the influence of aggressive environment and enzymes of the gastrointestinal tract.
- The presence of a mucosal barrier protecting the epithelial wall. This problem can be solved by developing an enhancer that will facilitate the penetration of insulin through the protective barrier.

A novel copolymer hydrogel of methacrylic acid polyacrylate-co-hydroxyethyl methacrylate-co-methylacrylate (MAA-co-HEMA-co-MA) was prepared and dissolved in methylcellulose (MC) to deliver insulin as a rectal suppository. Glycaemic levels in diabetic rats administered the binary hydrogel containing insulin showed a significant reduction in blood glucose levels up to 7.8 mmol/L (the effect persisted after 8.5 h) compared to subcutaneous administration of insulin, resulting in a reduction of 10 mmol/L within 4.5 h. Insulin concentrations when administered rectally ranged from 5 to 20 IU/kg over 24-48 h with a release efficiency of 75.9%. In addition, multiple emulsions of eicosapentaenoic, oleic or docosahexaenoic acids may be the insulin carrier in this delivery method. For rectal delivery, docosahexaenoic acid improves insulin permeability by acting as an enhancer.

Methods of insulin delivery using micro-needles

Microneedling is a minimally invasive technique that can be used to administer insulin. Micro-needles are designed to allow them to penetrate the stratum corneum of the skin for rapid drug release without causing permanent damage to the skin. Currently in the preclinical research phase, micro-needles fall into several categories depending on the type and materials from which they are made. For example, there are solid micro-needles to create an opening large enough to deliver small molecules and proteins, including insulin. The best mechanical stability was achieved with 600 μm long micro-needles compared to 700 and 800 μm. The penetration percentage of 600 μm needles remained at 90% even after multiple injections. As for the 700 and 800 μm diameter micro-needles, the number of successful attempts decreased to less than 20%. This may be due to insufficient mechanical properties due to the longer length. An in vivo absorption study in rats pre-injected with insulin via micro-needles showed a reduction in blood glucose levels to 29% of the initial 100% after 5 h. An alternative is the use of dissolving micro-needles, where the drug is encapsulated in a soluble matrix when injected into the skin. For example, when hyaluronic acid was used, such micro-needles dissolved completely 1 h after application. In biodegradable micro-needles, drug release from the matrix is controlled and can be maintained over a long period. For a stable hypoglycaemic effect, high concentrations of insulin are used in the matrix of such needles, which is due to the duration of the process of transition of the molecule from the depot to the systemic bloodstream, which can be considered both an advantage and a limitation of the method. Thus, biodegradable microneedling is a potential treatment option for DM with better results in maintaining serum insulin levels compared to subcutaneous administration.

‘Smart’ insulins

‘Smart’ insulins Since the 1970s, scientists have been trying to develop “glucose-sensitive” insulin preparations, and many patent applications have been filed and approved, but none of these developments have yet been approved for clinical use. Most published work has used encapsulation of insulin in polymers stored in subcutaneous depots and some mechanism to stimulate a response to an increase in blood glucose levels. However, these systems are usually too slow to respond to changes in glucose levels, making them unsuitable for clinical applications. In 2010, the bioactivity of oligosaccharide insulins was found to be dependent on blood glucose levels. However, Phase I clinical trials failed to produce conclusive results and the programme was discontinued.

Insulins with hepatic mechanism of action

Hepatopreferential insulin has been proposed as an alternative to restore the physiological ratio of portal to peripheral insulin and to reduce the risk of hypoglycaemia and minimise weight gain. Insulin peglispro is a molecule consisting of a polyethylene glycol chain covalently linked to the B28 lysine of insulin lispro and exerts hepatopreferential effects due to its large hydrodynamic size. Despite its potentially greater efficacy, insulin pegpro was associated with an increase in liver fat and triglycerides and a higher incidence of increased aminotransferase levels. Although this was not associated with severe liver damage, the manufacturer decided to stop the development programme in 2015. Another interesting development is an oral hepatocyte-directed vesicular insulin based on lipid nanoparticles. Phase II/III clinical trials were completed in 2009. According to the results obtained, against the background of therapy with the new type of insulin there was a significant decrease in the mean area of postprandial plasma glucose under the curve (AUC) compared to placebo without increasing any safety risks. However, there was no expected dose-dependent response when insulin concentrations were increased. The company has since changed the drug's prescribing for use in injections and infusions and achieved good results in glycaemic control in DM1 patients in the Phase IIb clinical trial NCT02794155.