Insulin Structure, Function and Diabetes Models in Animals

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ABSTRACT

Insulin is a peptide hormone that is synthesized and secreted by beta cells in the Langerhans islets of the pancreas. It regulates carbohydrate, fat, and protein metabolism by providing glucose in the blood to pass to fat, liver and skeletal muscle cells. A metabolic disease called diabetes occurs as a result of inadequate insulin activity in the body or the deterioration in insulin secretion in beta cells. Therefore, the discovery of insulin has been a revolutionary milestone in understanding both the treatment and prognosis of diabetes.[1,2]

The discovery of insulin

Insulin is derived from the Latin word insula, meaning island. In 1869, Paul Langerhans[3] found an unspecified cell cluster in the pancreas other than exocrine hormones, and in the coming years, many scientists prepared pancreatic extracts to lower blood sugar and reduce glycosuria in experimental animals. However, in 1921, the pancreatic extract was not purified until the pancreatic extract applied by a group of scientists, including Canadian orthopedic doctor Frederick G. Banting and his assistant Charles Best,[4-6] and insulin hormone was identified with this discovery. The molecular structure of insulin was discovered in 1955 by Frederick Sanger[7] by sequencing the amino acid sequence of pig insulin by primary sequence determination.[1] Hexameric 2-Zn insulin (T structure 6), which is the 3D structure of insulin, was determined by Dorothy C Hodgkin et al. in 1969 by X-ray method.[8]

The structure of insulin

Insulin has 51 amino acids and 6000 Da molecular weight in almost all species, including human.[9] The human insulin molecule consists of two polypeptide chains, one A chain and one B chain containing 21 and 30 amino acid residues, respectively.[10] These two chains are interconnected by SS (CysA7-CysB7 and CysA20-CysA19) with a disulfide bond, and an additional disulfide bond connects CysA6 and CysA11 in chain A (Figure 1). The amino acids of the two chains also participate in many non-covalent interactions.[9]

Although insulin binds to the insulin receptor in the form of a monomer, it turns into different
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Insulin forms in physiological or solution form.\textsuperscript{11,12} Formation of dimer, double dimer (tetramer), triple dimer (hexamer) or higher grade aggregates can be observed.\textsuperscript{13} Form of insulin in solution; It varies depending on insulin concentration, pH, solution composition and metal ion concentration. This also affects the charge and solubility of the insulin molecule.\textsuperscript{11,13} Monomeric human insulin molecules in solution at physiological pH are only present in very low concentrations (<1×10\(^{-9}\) M). The dimer is formed at a concentration of 1×10\(^{-6}\) M.\textsuperscript{11,13} In the blood, the physiological concentration of insulin is <1×10\(^{-9}\) M, and it provides its biological effect by participating in the insulin circulation in the form of a monomer.\textsuperscript{11} In case of increased concentration, insulin collection is initiated and several models have been proposed to identify the case in the absence of metal ions.\textsuperscript{13,14} In the presence of zinc or other metal ions; three human insulin dimers easily combine with hexamer.\textsuperscript{13,15} 2Zn hexameric insulin is collected in the coordination of two zinc ions and has a structure similar to the natural storage types of the peptide in pancreatic beta cells.\textsuperscript{15} This structure also prevents the formation of higher grade aggregates. The monomer form of insulin is active; The complexes formed are not active, but they can be progressively cleaved to release active proteins (Figures 2). This feature is used to create long-acting insulin medical formulations.\textsuperscript{16,17}

Although the amino acid sequence of insulin varies among the species, the positions of the three sulfur bonds, some parts of the molecule, both ends of the A chain, and the C-terminal residues of the B chain are highly preserved. This similarity leads to a three-dimensional form of insulin, which is very similar across species, and insulin from an animal is likely to be biologically active in other species. Until the discovery of synthetic human insulin, swine and bovine insulin were used in the treatment of diabetes.

Figure 1. Primary structures of swine insulin and swine proinsulin. Human insulin is similar to swine insulin except for the exchange of Ala\(^{B30}\) and Thr\(^{B30}\).

Figure 2. (a) Insulin hexamer and (b) Insulin monomer crystal structures.\textsuperscript{19} Insulin monomer structure is active hormone and hexamer structure is storage form. (A) Each of the 6 monomers is shown in a different color.
Pig insulin differs from human insulin in the B chain; Threonine, the 30th amino acid in human insulin, was replaced by alanine in pig insulin (Figures 2). Bovine insulin differs in both A and B chains. Threonine which is the 8th amino acid in chain A of human insulin is replaced by alanine, the 10th amino acid isoleucine by valine, and the 30th amino acid threonine is replaced by alanine in chain B. Due to these structural differences, there is a higher risk of developing local and systemic reactions, lipodystrophy and antibody at the injection site, especially against bovine insulin.[18,19]

**Biosynthesis of insulin**

Insulin is the biosynthetic product of preproinsulin, a single-chain precursor due to the exchange of proteolytic processes between cellular compartments. Biosynthesis of insulin; It begins by converting insulin mRNA into preproinsulin, a single molecule of 110 amino acids. After this structure passes through the endoplasmic reticulum, the signal consisting of 24 amino acids under the enzyme effect is separated from the peptide structure and transformed into proinsulin structure by folding and binding. Then proinsulin passes into the golgi apparatus and is exposed to several specific endopeptidases that exclude the C peptide (one of the three domains of proinsulin) consisting of 33 amino acids and thus produce the mature form of insulin. Insulin is secreted from the cell by exocytosis and spreads to the islet capillary blood. The C-peptide is also secreted into the blood at a 1:1 mole ratio with insulin. Although the C-peptide did not provide a biological detection, it is used as a useful marker for insulin secretion.[20-22]

**Insulin receptor**

The insulin receptor is a member of the transmembrane signal proteins of the tyrosine kinase family and is synthesized as a single polypeptide. The formed polypeptide is glycosylated and divided into alpha-beta subunits, and a tetramer is formed, which is bound by disulfide bonds again. The hydrophobic part of each subunit is located within the plasma membrane. Alpha subunit contains the insulin binding site. The cytosolic part of the beta subunit is a tyrosine kinase and is activated by insulin. Binding of insulin to alpha subunits of the receptor causes positional changes (Figures 3).[11] These changes are transmitted to beta subunits, and the tyrosine unit in these structures causes autophosphorylation. Consequently, insulin-receptor substrates (IRS-1 and IRS-2) activate phosphorylated phosphoindylinositol-3 kinase (PI3K) and mitogen activated protein kinase (MAPK) pathways. Eventually, insulin acts on the target tissues.[23-25]

**The function of insulin**

Insulin provides glucose homeostasis by keeping the plasma glucose value in an optimal range throughout the day. The main effects of insulin are: (i) In the liver, to stimulate glucose oxidation and storage of glucose (glycogenesis), as well as to convert glucose into triglycerides and protein synthesis, (ii) in the muscle tissue, it provides glucose uptake into the cells, and be stored as glycogen, (iii) and in fat tissue, it provides glucose uptake and conversion to triglycerides for storage.[22,26]
DIABETES MELLITUS

Diabetes mellitus, also known as diabetes, is a metabolic disease characterized by insulin secretion, insulin effect, or hyperglycemia as a result of the disorder observed in both. There are two main types of diabetes: Type 1 and Type 2 diabetes. Type 1 diabetes is caused by impaired insulin secretion due to damage in β cells, while Type 2 diabetes; It is caused by increased insulin resistance to the effect of insulin or a decrease in insulin secretion from the pancreas.\[27,28\]

Diabetes may cause disability and death in the long term by causing dysfunction and damage to the eyes, kidneys, nerves, heart and blood vessels. For this reason, it is common to use experimental animal models to understand the pathogenesis of the disease and to examine the prevention and treatment opportunities.\[27-29\]

EXPERIMENTAL ANIMAL MODELS

In experimental animals, experimental diabetes formation can be done by using chemical agents, spontaneous or virus.

1-Diabetes models created by using chemicals

In order to trigger type 1 diabetes, the most common streptozotocin (STZ) or alloxane is used, although zinc phillators (dithizone, 8-hydroxyquinoline), Rodenticide-Vacor, dietary nitrosamines are used. These compounds are similar in structure to glucose and animals are more susceptible to hypoglycemia, as glucose competes with STZ and alloxan. In addition, these substances have an unstable structure, so the formation of the solution should be done just before injection.\[30-32\]

STZ and alloxan are also used to create Type 2 diabetes in animals.\[31\]

Streptozotocin (STZ)

This structure, also called Streptozosin, is synthesized by the fungus Streptomycetes achromogenes. Its chemical structure is [2-deoxy-2- (3- (methyl-3- nitrosoureido) -d-glycopyranose]. It enters beta cells with glucose in its structure with GLUT 2, causing alkylation of beta cells in DNA bases. (ADP-ribose) polymerase (PARP) discharges NAD stores and reduces ATP content using nicotinamide adenine dinucleotide (NAD) in the cell, thereby causing necrosis in beta cell.\[31,33\]

STZ should not leak out of the vein as it can irritate tissues. In addition, it should be used by dissolving in a citrate buffer solution with a pH of 4.5.\[31\]

STZ can be given as a single high dose or multiple low doses. It has been observed that 100-200 mg/kg of mice given high doses at once and 35-65 mg/kg of rats cause diabetes.\[31,34,35\] Multiple low doses range from 20 to 40 mg / kg per day, depending on the species and strain. Multiple low doses can be given for 5 days in rats and mice.\[31,36,37\]

Alloxan

Alloxan, 2,4,5,6-tetraoxypyrimidine; It has 5,6-dioxouracil structure and its diabetic effect is due to free radical formation in beta cells. Alloxan is reduced to diraluric acid and oxidized repeatedly, and a redox cycle is formed for the formation of superoxide radicals, especially the hydrogen peroxide radical. At the end of the reaction, highly reactive hydroxyl radicals are formed which cause beta cell DNA to break down.\[31,38\]

Alloxane 50-200 mg/kg in mice and 40-200 mg/kg in rats is sufficient, while it can be administered intraperitoneally (i.p), subcutaneously (s.c), intravenously (i.v).\[31,38\]

2-Spontaneous diabetes models

Autoimmune models used in Type 1 DM; non-obese diabetic (NOD) mouse, Bio reproductive (BB) rat, LEW.1AR1 / Ztm-iddm rat.\[39,40\]

The NOD mouse is a lab mouse developed in Osaka in 1974.\[41\] Insulitis development is observed in pancreas when it is 3-4 weeks old and diabetes develops when it is 30 weeks old.\[42\] The rate of diabetes differs in genders and it is more likely to be seen in females.\[42\] While the Type 1 diabetes genes in humans are parallel with NOD mice, some pathways and mechanisms are different.\[40\]

Therefore, there are many drugs that work in these mice but not in humans. Also, diabetes in NOD mice is negatively associated with microbial exposure, so it should be kept in specific pathogen-free conditions (SPF).\[31\]

Bio reproductive rats were obtained from the deceased Wistar rats and were first described in Canada in 1974. BB rats generally develop diabetes at puberty and the incidence is the same in men and women. Approximately 90% of rats develop diabetes when they are 8 to 16 weeks old. Like NOD mice, insulitis develops, whereas lymphopenia is not observed only in humans and NOD mice, while lymphopenia is observed in BB mice. This factor appears to be a disadvantage for the use of BB rats as a model in Type 1 DM.\[31,43\]
LEW.1AR1/-iddm mice were raised in an MHC haplotype congeneric Lewis rat colony raised at the Hanover Medical School Laboratory Animal Science Institute. In these rats, insulitis is observed and diabetes occurs in 8-9 weeks. It is observed equally in both genders. Unlike the NOD mouse and BB rat, it does not exhibit any other autoimmune disease.[31,41,44]

Most of the type 2 diabetes animal models are obese and the classification is made accordingly. Among the monogenic obese models, Lep ob/ob mice, Leprdb/db mice, Zucker Fatty rats (ZDF); Examples of polygenic obese models are KK mice, OLETF rats, New Zealand obese (NZO) mice, TallyHo/Jng mice, NoncNZO10/Ltj mice. There are also non-obese animal models and Goto-Kakizaki rats, hiAPP mice are examples.[31]

In obese type 2 diabetes animal models that are obese, Lep ob/ob mice[45] are caused by a lack of saturation-promoting leptin hormone and in Leprdb/db mice,[46] obesity is observed due to leptin hormone receptor deficiency.

Lep ob/ob mice create a severe obesity model, while weight gain begins at 2 weeks of age, and mice develop hyperinsulinemia. In addition, these mice are sterile.[45]

Leprdb/db mice originate from an autosomal recessive mutation discovered in Jackson Laboratory. When 4-8 weeks of age, hyperglycaemia is observed and ketosis develops after a few months and is short-lived.[31,46]

3- Diabetes models created by virus

Most of the encephalomyocardine virus was studied with the M variant in the diabetes models created by the virus. This virus is from the picarnovirus family and as a result of injection, insulitis is observed. Stubborn hyperglycemia, ketoacidosis is observed and results in death. KRV, Coxsachie B, Rubella, Reovirus, CMV are other viruses used to create animal models of diabetes in animals.[47]

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