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Diabetic Relief in a Glass: Cow's Milk Engineered to Produce Human Insulin

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Diabetes is a chronic metabolic disorder characterized by high levels of glucose in the blood. It occurs either due to insulin deficiency or insulin resistance severely damaging several organs. Insulin is a hormone secreted from the beta cells of the islets of Langerhans in the pancreas that helps regulate blood sugar levels and allow glucose to enter cells, where it is required for energy. Insulin is a protein consisting of two polypeptide chains, one of 21 amino acid residues and the other of 30, joined by two disulfide bridges. It is a key player in the control of intermediary metabolism. It has profound effects on both carbohydrate and lipid metabolism and has a significant influence on protein and mineral metabolism. Disturbances in insulin level or responses have widespread and devastating effects on many organs and tissues. Inadequate lowering of blood glucose level to normal level results in diabetes. Generally, people have a fasting sugar level less than 110 mg/dl, which is considered normal. Diabetes is either type 1 or type 2.

Type 1 diabetes occurs due to the autoimmune destruction of beta cells in the pancreatic islets. As a result, the body produces little to no insulin and increases the blood and urine glucose. This type of diabetes typically develops in childhood or adolescence, although it can occur at any age. People with type 1 diabetes require lifelong insulin therapy to manage their blood sugar levels. Type 2 diabetes is the most common form of diabetes, accounting for the majority of cases worldwide. In this type, hyperglycemia is due to the progressive loss of insulin secretion by beta cells against a background of insulin resistance. To compensate for insulin resistance, a high amount of insulin is secreted, which in turn increases blood glucose levels. Other types of diabetes include gestational diabetes, which occurs during pregnancy, and various forms of diabetes caused by specific genetic mutations, diseases, medications, or pancreatic disorders.

Diabetes is a significant global health concern, with millions of people affected worldwide. The number of people with diabetes worldwide has doubled during the past 20 years. It was estimated that 537 million people had diabetes in 2021 and, this count is projected to reach 783 million by 2045. Individuals with diabetes face an increased risk of cardiovascular disease, kidney failure, nerve damage, blindness, and even lower limb amputations.

Biosynthesis of Insulin

Insulin being an essential hormone, is synthesized in the body by a complex process orchestrated primarily by the beta cells within the pancreatic islets. It begins with the transcription of the insulin gene (INS) into mRNA, which serves as a template for protein synthesis. The mRNA undergoes processing, to form mature mRNA. Within the cytoplasm, ribosomes translate the mRNA sequence, assembling amino acids into preproinsulin. This preproinsulin undergoes further modifications as it moves into the endoplasmic reticulum (ER), where a signal peptide is cleaved to form proinsulin by signal peptidase enzyme. In the ER and Golgi apparatus, proinsulin undergoes folding and post-translational modifications, including the formation of disulfide bonds and proteolytic cleavage. This results in the packaging of prohormone into secretory granules by prohormone convertases (PC1/3 and PC2) and carboxypeptidase E, generating two mature insulin and C-peptide. Upon stimulation by elevated blood glucose levels, these granules fuse with the cell membrane, releasing insulin into the bloodstream, where it regulates glucose uptake and utilization in various tissues throughout the body. Any disruption in this intricate process can lead to impaired insulin production and secretion, contributing to the pathogenesis of diabetes mellitus.

Insulin therapy plays a crucial role in the management of diabetes

During the early part of the 20th century, before insulin became available, fasting and calorie-restricted

diets were practiced. All diabetics were advised to decrease their sugar and dietary starch intake, and those who were obese were advised to lose weight. This resulted in some improvement in glucosuria and acidosis.

The discovery of insulin marked a major breakthrough in medicine and therapy in patients with diabetes. It was isolated in 1921 with its first clinical use in 1922. Insulin therapy is essential for individuals with type 1 diabetes, as well as many people with type 2 diabetes who require insulin to achieve optimal blood glucose control. It aims to mimic the natural release of insulin by the pancreas. Insulin can be produced through various methods, including traditional extraction from animal sources, recombinant DNA technology using genetically engineered microorganisms, and more recent advancements such as human stem cell-derived insulin. Historically, insulin was extracted from the pancreas of animals, particularly pigs and cows. The pancreas glands were harvested, processed, and purified to isolate insulin. This method has been replaced by recombinant DNA technology due to concerns about purity, consistency, and potential for allergic reactions.

Genetically modified organisms (GMOs) are living organisms whose genetic material has been altered or modified using genetic engineering techniques. This manipulation involves the insertion, deletion, or modification of specific genes to introduce desired traits or characteristics into the organism. GMOs can be found in various fields such as agriculture, medicine, and industry. In industry, microorganisms are often genetically modified to produce enzymes or chemicals for various applications. Recombinant DNA technology revolutionized insulin production by allowing for the production of human insulin using microorganisms, typically Escherichia coli or Saccharomyces cerevisiae. Using this method, insulin has been produced using different strategies including the separate production of the A and B peptide chains and later recombined to form biologically active insulin, biosynthesis as proinsulin or as mini-proinsulin and proinsulin.

In animals, human proinsulin was produced in the milk of transgenic mice and converted to insulin in vitro using the proteases trypsin and carboxypeptidase B. Transgenic animals are considered potential candidates for recombinant protein production on a large scale. Milk has more advantages for the production of recombinant proteins. The Expression of heterologous proteins in milk can be manipulated using different mammary gland-specific promoters, and the proteins produced are secreted, which may facilitate or complicate their purification. But even with these advantages, the concern is the high cost and time of generation of a transgenic animal. The vector constructed for transgenesis is evaluated before an animal is generated. Research studies have explored the production of heterologous proteins in the mammary glands of transgenic animals, including posttranslational modification. Due to their ability to produce abundant quantities of milk over extended lactation periods, cows can be viewed as valuable bioreactors for the expression of recombinant proteins in milk.

Engineering Cows to Produce Human Insulin

At present, biopharmaceuticals derived from the milk of transgenic animals are being commercially developed for various human health purposes. The mammary gland is a tissue where posttranslational modifications are possible for large-scale recombinant protein production. Examples include antithrombin-α (ATryn) produced in goat milk, C1 esterase inhibitor (RUCONEST) manufactured in rabbit milk, and a range of other therapeutic proteins. Moreover, besides biomedical protein production, transgenic bovines have been engineered to alter nutritional components in animals intended for human consumption. This includes modifications to proteins, amino acids, and lipids, to produce safe food products for human consumption, such as eliminating allergenic proteins from milk. Additionally, transgenic cattle have been developed to enhance disease resistance, further advancing the potential benefits of biotechnology in agriculture and medicine.

In this process, transgenic animals containing a transgene are produced, which drives recombinant

protein expression in a tissue-specific manner in the mammary gland. To test this gene, special cells (MAC-T) are used to mimic mammary cells. The cells are grown in the lab and exposed to lactogenic hormones that stimulate human insulin (hINS) protein production. These cells serve as a quick way to check if the gene works before making the transgenic animals. Everything is checked before the long process of creating genetically modified livestock and waiting for them to produce the protein in their milk. These cells are a useful tool to confirm that the gene is doing its job correctly.

To ensure that all offspring produced are transgenic, Somatic cell nuclear transfer (SCNT) is used. It has proven successful in generating transgenic livestock species, including sheep, cattle, and swine. After manipulation, transfection, or transduction of cell lines, and subsequent in vitro screening, only the modified cell lines are selected as nucleus donors to produce embryos. These transgenic blastocysts can then be transferred to recipients to generate transgenic cloned animals. However, SCNT for generating transgenic cows has shown low efficiency, with only a single calf obtained at the end of each pregnancy, and an extended period required for recombinant protein expression. Nevertheless, promising methodologies have been employed to overcome the low efficiency of SCNT.

For the production of insulin in cow milk, the lentiviral heterologous gene expression technique has been used. In this study, a lentiviral mammary gland-specific expression vector was constructed to contain the human insulin gene driven by the bovine beta-casein promoter. Bovine fibroblasts and immortalized mammary epithelial cells (MAC-T cells) were transduced with this lentivirus vector. The evaluation of the vector constructed was done in vitro. Modified bovine fibroblasts were used for SCNT to generate transgenic calves expressing human proinsulin in milk.

The presence of recombinant protein was confirmed by evaluating the milk samples of non-transgenic and transgenic cows on the 23rd day of lactation using mass spectrometry. In this analysis, it was possible to identify a peptide corresponding to the C-peptide from human insulin that differentiated in mass from bovine insulin. It was reported, that the C-peptide found in the mass spectrometry was composed of 28 amino acids, differing from the 31 amino acids. The identification of the C-peptide confirmed the presence of the insulin in cow's milk and proinsulin could be converted into insulin by milk proteases or by use of trypsin treatment for mass spectrometry analysis.

Paulo S. Monzani used the combination of lentivirus and SCNT gene transfer methodology to successfully generate a transgenic calf that contains the gene to produce human proinsulin in milk. In this way, a large amount of insulin can be produced for a longer period. The recombinant protein can be easily purified from milk giving another advantage. This is an exciting system that can help millions of diabetic patients by providing insulin therapy.

Conclusions

Using transgenic animals as bioreactors to produce pharmaceutical proteins is proposed as an efficient and cost-effective method. The mammary gland is ideal for large-scale production of recombinant proteins, as it can perform posttranslational modifications. The combination of gene transfer mediated by lentivirus and SCNT methodologies used in this work successfully generated a transgenic calf that contains the gene to produce human proinsulin in milk. This breakthrough in insulin production offers hope for millions living with diabetes mellitus. By streamlining the production process and reducing costs, this method has the potential to improve access to affordable insulin therapy. This, in turn, can empower countless individuals to manage their diabetes more effectively and lead healthier, fuller lives.

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