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Potential of pramlintide on T1DM treatment

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Abstract. T1DM is an autoimmune disease that has CD4+ and CD8+ cells attacking islet ß cells. The destruction of islet ß cells disrupts the ability to produce insulin severely, causing hyperglycemia and insufficient energy production, leading to symptoms such as drowsiness and body weakness. Excessive bloodstream glucose is discharged through urination. While the trigger of this autoimmunity is unknown, HLA class II haplotypes HLA-DR3-DQ2 and HLA-DR4-DQ8 on chromosome 6 are thought to be responsible for T1DM development. Though T1DM incidence rate varies among countries, patients under 15 years old are drastically increasing. While insulin is the most well-established current treatment of T1DM, amylin helps to increase the effectiveness of glucose regulation by insulin. In pramlintide, amino acids 25, 28 and 29 are different from amylin, which solves the problem of amylin being self-aggregating and insoluble after injection. However, the injection dose of pramlintide needs to be carefully controlled to prevent hypoglycemia. Responding to the increasing number of T1DM patients, pramlintide increases the effectiveness of insulin treatment.

Keywords: T1DM treatment, pramlintide, amylin.

1. Introduction

Type 1 diabetes has a long history in human civilization. It was first recorded in ancient Egypt, ancient China, ancient India and Greek and Arab medical documentations. The official name of diabetes was nailed down in the 2nd century by Aretaeus of Cappadocia, providing a detailed description about diabetes for the first time, In the 17th century, Thomas Willis invented the term “mellitus”, which describes the sweet urine from diabetes patients [1]. French physiologist Claude Bernard investigated the glycogenic action of the liver in the 19th century. In the same century, Oskar Minkowski and Joseph von Mering experimented on removing pancreas in dogs, discovering the removal of pancreas causes diabetes. Charles Best and Frederick Banting successfully purified a sample of insulin out of pancreas, and they successfully treated diabetes patients with insulin, discovering the drug for diabetes treatment [2].

There are two types of diabetes, the T1DM and T2DM, but the typical symptom for both types is having a high blood concentration. For this paper, only T1DM is discussed. In a normal glucoregulation process, insulin is needed to bind to cell receptors to allow glucose in the bloodstream enter body cells. However, for T1DM patients, they cannot produce insulin spontaneously, meaning that glucose will remain and accumulate in the bloodstream, and only very little of glucose can enter body cells. Other symptoms of T1DM include polydipsia, frequent urination, unexpected weight loss, drowsiness, etc. While glucose keeps accumulating in the bloodstream, the human body attempts to remove the excessive glucose in our body. As a result, it becomes the kidney’s job to remove the extra glucose through

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urination. This is the reason why patients with T1DM have urine that is sweet [3]. Because glucose is removed through urination, the brain signals the body to drink more frequently to accommodate the need of removing extra glucose. Fatigue is also one of the most prominent symptoms in T1DM. Because glucose cannot enter body cells, body cells cannot properly produce energy through glycolysis [4]. As a result, less energy is produced in the absence of glucose in body cells, which thus make T1DM patients fatigue. Because not enough energy is produced, the brain assumes the body is starving. As a result, the body consumes muscles and fats for more energy.

There are three stages of diabetes progression [5]. In stage one diabetes, ß-cell destruction and autoimmunity can already be detected, but there is no hyperglycemia, nor any symptoms of diabetes. In stage two diabetes, while hyperglycemia is present, there are still no symptoms caused by glucose accumulation in bloodstreams. In stage three diabetes, symptoms of diabetes start to emerge. As a result, stage one and two diabetes are presymptomatic, and stage three is symptomatic. Most of the patients are diagnosed with stage three diabetes. Of all patients diagnosed with diabetes, only 10 to 15 percent of all patients are in type one. 85 to 90 percent of all patients are in type two diabetes. However, T1DM is the most prevalent type of diabetes in children below 15 years old. Because T1DM is considered as a genetic rare disease, whereas the T2DM can be developed postnatally, T1DM is relatively rare in the diabetes population [6]. According to Katsarou et al., the incidence rate of T1DM peaks in the age interval of 10-14, and declines after this age interval [6] (Figure 1). However, the incidence rate might peak at an earlier age interval, due to the fact that some patients might have T1DM much earlier than they have been diagnosed with it. According to an observational study of T1DM patients since birth, their first autoantibody developed between 6 months to 24 months of age. When they grow to 2-4 years old, their autoantibody increases from one to multiple, followed by stage three T1DM in the future. Also, the incidence rate for ages younger than 5 is increasing drastically, and the incidence rate for age intervals 5 to 15 increased by 70 percent in recent years. Interestingly, the peak of incidence rate appears to be earlier in girls than boys. This is speculated to be caused by the earlier puberty development in female population [6].

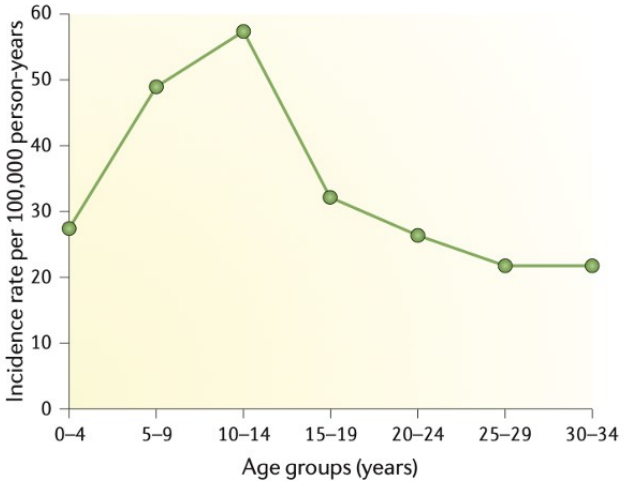


Figure 1. T1DM incidence rate of different age intervals [7].

For the incidence rate worldwide, the number of patients is very different between countries (Figure 2). For Asian countries, such as China, Korea and Japan, T1DM is relatively rare. However, in Scandinavian countries and European countries, the incidence rate is much higher compared to Asian countries [6]. The reason for this variation is still in study, but it is known that this variation is due to environmental factors and genetic factors. For environmental factors, hygiene and lifestyle can affect the T1DM incidence rate in a specific population. For genetic factors, since it is known that T1DM is attributed to mutations on HLA gene, the HLA gene risk factor of a specific population can affect the incidence rate of T1DM. Because the HLA genes are very different between each individual, there might

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be a population that has a significantly higher number of mutated HLA gene individuals, leading to potentially more T1DM in this population [6]. Also, some African countries appear to have a lower incidence rate, but the actual incidence rate might be higher than what we observed because the lack of technology made many potential T1DM patients yet to be diagnosed. From the graph, interestingly, the incidence rate of T1DM is higher in developed countries and lower in developing countries. Diabetes is often referred to as a "rich country disease".

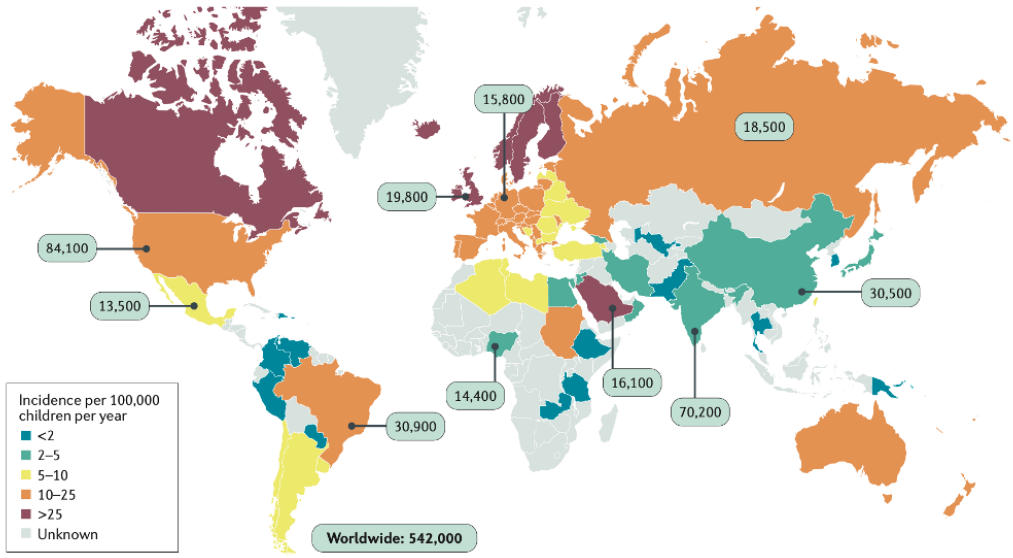


Figure 2. T1DM incidence rate of different countries [7].

Currently, the most prevalent treatments of T1DM include insulin injection, islet transplantation and stem cell therapy [7]. Because T1DM patients cannot produce insulin spontaneously to accomplish glucose regulation, insulin can be injected to the bloodstream and help glucose enter body cells [8]. Islet transplantation is much more restricted compared to insulin injection [9]. In T1DM patients, their islets responsible for producing insulin are destroyed by autoantibodies. As a result, transplanting a new islet can allow the body to regain the ability of producing insulin. However, it is very hard to find a matching islet transplant. It is also uncertain whether an islet can adapt into the new body. Stem cell treatment is recently investigated [10]. Stem cells can become any kind of cell in our body. After introducing stem cells into islets and adding the stem cell factor to signal stem cells to become islet cells, the effect of this therapy will accomplish the same effect of islet transplant in a more practical way.

2. Pathogenesis of T1DM

Though the exact trigger of autoimmunity is unknown, it is known that T1DM is an autoimmune disease attacking the islet ß cell in pancreas [11]. Scientists have speculated that the cause of this autoimmunity is because of mutations on the HLA gene. Specifically, the HLA class II haplotypes HLA-DR3-DQ2 and HLA-DR4-DQ8 on chromosome 6. Somehow the immune system will see islet ß cells as foreign and detect insulin as antigens. The B cell will detect insulin as an antigen, and signals CD4+ and CD8+ cells to attack islet ß cells. Dendritic cells are also involved in this signaling of T cells [12]. There are also autoantibodies produced by the B cell, which will bind to the islet ß cells, significantly reducing their ability to reproduce. There might also be mutations on the HLA gene that is responsible for the lack of T regulatory cells [13]. Once the autoimmune T cells emerge, there are somehow no or very little of T regulatory cells in T1DM patients, eventually leading to the destruction of islet ß cells (Figure 3).

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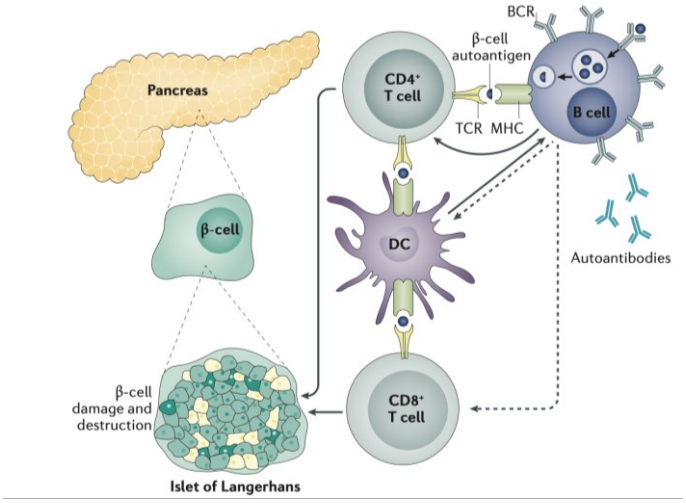


Figure 3. Pathology of T1DM [7].

3. Potential of pramlintide treatment on T1DM

Pramlintide is an amylin analog that has potential on T1DM treatment. Amylin is a hormone that is co-secreted with insulin [14]. While insulin helps glucose to enter body cells, amylin is also secreted in pancreas and finally binds to the brain, which will cause the brain to secrete other kinds of hormones. Those other kinds of hormone will signal the liver to suppress postprandial glucagon secretion and signal the stomach to reduce food intake and appetite [15]. In other words, while insulin helps glucose to enter the body cell, amylin helps to further reduce glucose level in our body through controlling liver glucagon secretion and reducing food intake (Figure 4). As a result, implementing both insulin and amylin injections can increase the effectiveness of reducing bloodstream glucose level [16]. However, some features made amylin injection impractical. First, amylin is proved to be insoluble and self-aggregating in the bloodstream, limiting the ability of amylin binding to the brain [17]. Second, amylin is prone to self-aggregate in pancreatic tissues. Once they are aggregated in islet, they will damage islet tissue and ß cells to the point of functional failure [17]. As a result, a change in amylin structure is needed to make it a practical treatment on T1DM.

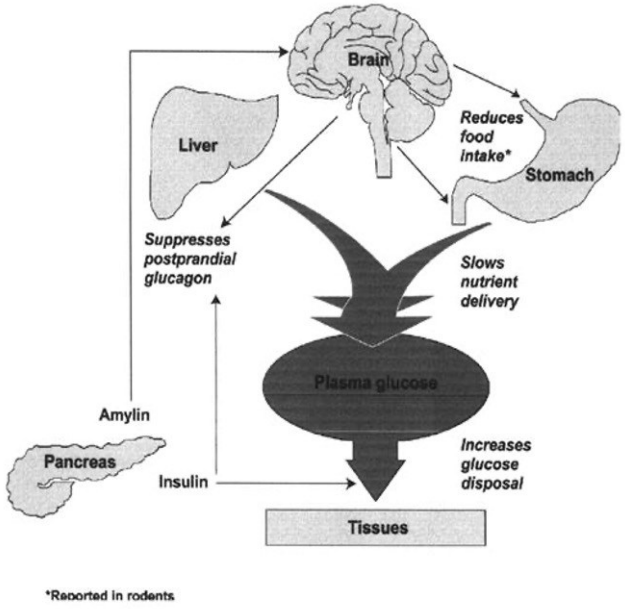


Figure 4. Mechanism of amylin [17].

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Pramlintide is very similar to amylin. Only three amino acids of pramlintide are different from human secreted amylin, which are the 25, 28 and 29 amino acids [17] (Figure 5).

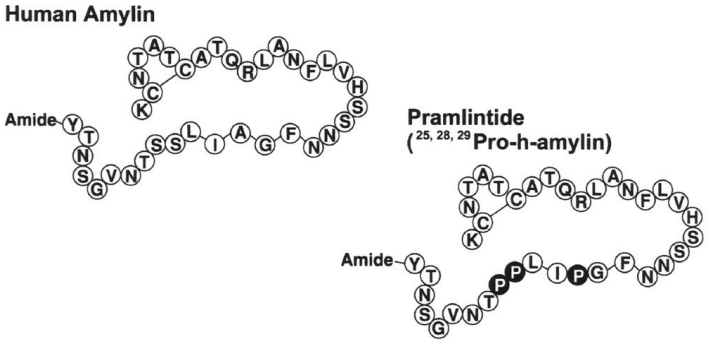


Figure 5. Structural difference between pramlintide and human amylin [17]

Originally, the amino acids on positions 25, 28 and 29 are two serine and one arginine. In pramlintide, these three amino acids are changed into proline. This change prevented pramlintide forming the fibril which human amylin potentially could form. Prolines create a kink in the protein structure of pramlintide, which limits its ability to form fibrils [18]. The rigidity of proline prevents beta sheets from forming in pramlintide, which is a prevalent structure in amylin fibrils. Proline's rigidity allowed pramlintide to be more stable in structure, increasing the efficiency of drug delivery. The change of amino acids thus solves the problem of amylin being insoluble and self-aggregating [18]. Pramlintide is soluble and non-aggregating in the human body [17].

3.1. Steps to obtain Pramlintide

Gene editing technology will be involved in pramlintide production. First, extract the gene of interest. Because the structure of pramlintide and amylin is so similar, IAPP can be used as the gene of interest which can be mutated to the pramlintide coding gene later. E. coli will be the duplication vector in producing pramlintide. After cutting a human IAPP gene, insert it into the E. coli plasmid. For the plasmid setting, a strand for antibiotic resistance is needed to separate the mutated bacteria from the wild type bacteria. ORI and promoter are needed to mark the starting point of the transcription segment. Finally, the MCS will be the gene responsible for producing pramlintide. Mutagenesis is used to designate point specific mutations on the codons coding for 25, 28 and 29, changing the codons for coding serine and arginine to codons coding proline. After separating the wild type and mutated bacteria, put the mutated bacteria into a centrifuge. The cell membrane will break apart and pramlintide produced by the mutated bacteria will be outside the cell now. Ion exchange chromatography is used to separate pramlintide from other proteins in the bacteria. Since pramlintide has a positive charge (pI=10.2), no tags are needed to facilitate the protein purification. Eventually, the SDS page will be used to check the protein purification. Pramlintide has a molecular weight of 3951.41 Da. Ideally, a dense strand should appear on the corresponding position of 3951.41 Da.

3.2. Delivery methods of pramlintide

Injection is the most effective method among all delivery methods [18]. By injecting amylin into the bloodstream, it can directly circulate through bloodstreams and bind to the brain, whereas inhalation or oral delivery will be ineffective because it takes longer for the amylin to arrive in the bloodstream. Another issue is that amylin denatures in the stomach. As a result, oral delivery method has proved to be ineffective.

4. Conclusion

Pramlintide injection is proven to be effective in glucose regulation of T1DM patients, especially patients with severe symptoms. However, for less severe patients, pramlintide injection might cause

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hypoglycemia, which will cause sharp dizziness and weakness. This is because the human body can produce very little energy in the absence of glucose. As a result, the dose of injection must be carefully regulated to prevent side effects. Further studies are needed to understand the trigger of T1DM autoimmunity. Other than insulin injection, stem cell therapy is also effective in treating T1DM patients. Compared to islet transplant, the number of transplants is very limited, but stem cells can be mass produced. As a result, stem cells have a higher availability, meaning more T1DM patients can be treated. Stem cell also provides a long-term solution for T1DM compared to insulin injection. Insulin needs to be injected frequently, but there will be less needed to inject if stem cells recover the islet's ability to produce insulin, reducing patients' pain from injection. While a stem cell is effectively recovering the number of healthy islets ß cells, studies are needed to prevent the autoimmune system from further attacking islet ß cells.

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