Plasmid Gene Therapy To Treat Type-1 And Type-2 Diabetes

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ABSTRACT:

Current treatments for Type-1 and Type-2 diabetes suffer from their demand for frequent injections to their drug interaction issues. Plasmid gene therapy for diabetes has recently gained popularity, where a gene of interest (GOI) is inserted into a bacterial plasmid and injected into the body to go through biological processes. The gene is then translated to increase insulin levels and regulate blood glucose (BG) levels. Using the search engines Google Scholar and PubMed, I found experiments that met my inclusion and exclusion criteria on the basis of plasmid gene therapeutic experiments on animals genetically similar to humans. Experiments with the genes of interest adiponectin (ADN), glucagon-like peptide 1 (GLP-1), and IL-4/IL-10 had consistent results lasting multiple weeks. A treatment using electrotransfer (ET) administration methods also had consistent results. These treatments have demonstrated that they may be better than current ones, like intensive insulin therapy (IIT), Metformin, and Ozempic. Further testing may show that plasmid gene therapy has a strong potential to treat both forms of diabetes in humans.

INTRODUCTION:

Diabetes is a chronic disease affecting how the body regulates blood glucose (BG) and is caused by a lack of insulin production in the body [1]. Diabetes comes in two forms: Type-1 and Type-2 [1]. Type-1 tends to develop at a younger age as it is a genetic disorder, while Type-2 is more prevalent in adults as it is a result of a lack of physical activity and a healthy diet [1]. Type-1 diabetes occurs when the human immune system incorrectly attacks the pancreatic β-cells that produce insulin [1]. Without insulin to facilitate glucose, BG levels rise, leading to hyperglycemia [1]. Hyperglycemia causes dehydration and frequent urges to urinate. Eventually, these complications will cause harm to vital organs including the heart [1]. In comparison, Type-2 diabetes is not caused by a flawed attack on pancreatic β-cells influenced by genes, but rather behavioral factors such as poor diet and lack of exercise [2]. BG levels reach a peak if the pancreatic β-cells are simply unable to produce enough insulin to keep them under control [2]. When BG levels are excessively high as a result, diabetes patients are at risk of hyperglycemia [1]. Diabetes exists in a variety of ways and has a variety of current treatments [3].

Popular treatments for Type-1 diabetes include intensive insulin therapy (IIT) where insulin is administered to the body to substitute the insulin that was never produced by the failing pancreatic β-cells [4]. IIT requires 4-5 self-checks for BG levels and 3-4 injections of insulin daily [4]. Although IIT has been proven to assist in lowering BG levels and reducing the risks of organ damage, 1 in 2 patients fail to improve under IIT [4]. The time, money, and dedication a Type-1 diabetic is expected to inject insulin throughout the day are difficult to maintain, and failure to monitor their decreased BG levels carefully puts patients at risk for hypoglycemia, the reciprocation of hyperglycemia. Hypoglycemia causes nausea, headaches, fatigue, and if left untreated for long enough, organ damage just like its counterpart, hyperglycemia [1].

Ozempic, an injection that produces sufficient amounts of insulin and regulates BG levels is a known treatment for Type-2 diabetes [5]. However, injections must be administered weekly,

which may make adherence difficult for patients [5]. On the other hand, one of the most popular treatments for Type-2 diabetes worldwide is Metformin, a medication taken daily that reduces the amount of glucose produced by the liver leading to decreased BG levels [6]. However, Metformin lacks effectiveness when in contact with other drugs prescribed for treating Type-2 diabetes, which is a significant problem for people with more severe Type-2 diabetes who may need more than just Metformin [6].

A different form of diabetic treatment has recently grown in popularity: using genes of interest (GOIs) in plasmid vectors. A plasmid is a circular molecule of DNA found in bacteria that is commonly used in biomedicine as a vector for the replication of genes of interest due to its versatility and reproduction capabilities [7]. This form of gene therapy has been tested commonly on rodents who share 97.5% of their working DNA with human beings [8]. By adding a GOI to a bacterial plasmid vector, the plasmid, if administered carefully, will ensure quick and effective replication of the GOI. This applies to both Type-1 and Type-2 diabetes because hormones that assist in insulin production such as adiponectin (ADN) will be replicated on their own inside the body. With fewer injections, the risks of hyperglycemia and hypoglycemia are reduced [9]. Similarly, glucagon-like peptide-1 (GLP-1) has been known for its potential in diabetes treatment for decades, but the dipeptidyl peptidase 4 (DPP4) enzyme denatures GLP-1 in the body [10]. Plasmids protect this GOI, ensuring that GLP-1 levels increase in the body, effectively treating diabetes [10]. Unlike Metformin, there are no clear drug interactions with this treatment [6, 10]. Although Ozempic is an effective GLP-1 treatment as well, it must be taken every week whereas plasmid treatments appear to only require administration every 2 [11] or even 3 weeks [10]. Scientists may even be able to utilize gene therapy to *prevent* diabetes, as they counter the autoreactive T-cells in Type-1 diabetes with helpful T-cells IL-4 and IL-10 as genes of interest [12]. Additionally, new methods of administration are being developed, such as electrotransfer (ET) [13]. With plasmids, we can see a new future in the realm of Type-1 and Type-2 diabetes treatments.

The objective of this study is to review the effectiveness of a variety of genes of interest to be carried in the plasmid vector and assert their utilities based on how they were tested on animal models.

METHODS:

The search terms I used were “Type-1 diabetes”, “Type-2 diabetes”, “plasmids”, “gene therapy”, and “gene of interest” in the search databases PubMed and Google Scholar.

My inclusion criteria were research that used animal models with similar genetic make-up to humans, involved bacterial plasmids as the form of gene therapy with any gene of interest, and specifically used plasmids for the purposes of researching how plasmids offer protection and sustainability in the body. I also allowed experiments that aimed to treat any form of diabetes.

My exclusion criteria were research that focused on advertising a gene of interest or strategy, used animal models with less genetic similarity to humans, or used gene therapy technology without bacterial plasmid vectors, such as CRISPR technology. I also excluded

research that met the above criteria for diseases that are not for treating diabetes or increasing insulin in the body.

RESULTS:

GOIs and Methods of Administration

Nan et al. and Fukushima et al. administered ADN using the plasmid vector pVAX1 for the former, but unspecified for the latter [9, 14]. They also both administered streptozotocin (STZ) into the mice in order to give them Type-2 diabetes, however, Nan et al. administered the plasmid into multiple cell lines of the tail vein including HeLa, HEK293, HepG2, HT22, and SK-Hep 1 while Fukushima et al. focused on administration to HepG2 cells only [9, 14]. Jean et al. and Kumar et al. both administered GLP-1, but Kumar et al. used a GLP-1/Fc fusion protein, including a fragment crystallizable receptor (Fc) antibody [10, 11]. In contrast, Jean et al. used therapeutic nanocomplexes for plasmid material (TNC) [10]. Kumar et al. aimed to extend the shortened lifespan of GLP-1 in the body by administering the plasmid into the mice's tibialis anterior muscle while Jean et al. administered the plasmid into the skeletal muscle [11]. In contrast, Martinenghi et al. and Ko et al. utilized plasmids to treat Type-1 diabetic mice models [13, 12]. Instead of focusing on a specific GOI, Martinenghi et al. engineered special DNA sequences that directly code for insulin and focused on the procedure of an ET [13]. Ko et al. implemented beneficial T-cells, IL-4 and IL-10 as GOIs to prevent Type-1 diabetes in susceptible mice [12].

Outcomes

Nan et al. observed that when the expression levels of mRNA were checked, the cell lines that were exposed to the ADN-encoded plasmid treated the Type-2 diabetes effectively by facilitating BG levels and increasing insulin [9]. The duration of these results remained consistent for around 2 weeks [9]. In comparison, Fukushima et al. observed that the untreated mice exhibited lower ADN levels than the treated mice, who demonstrated mRNA levels of ADN 10-15 times more than before in HepG2 cell lines, also persisting for about 2 weeks after treatment [14]. Moreover, Kumar et al. noted that the GLP-1/Fc plasmid lifespan increased to 2 weeks after radioimmunoassay (RIA) confirmed its presence in transfected cells [11]. Jean et al. observed that treated diabetic mice models demonstrated roughly quadruple the amounts of GLP-1 compared to the control mice [10]. Raised GLP-1 levels persisted for 24 days following the final injection [10]. Furthermore, Martinenghi et al. recorded that 17 of the 20 mice observed had detectable levels of mature insulin in their bloodstream for around 6 weeks and that the procedure kept the treated mice alive for about 10 weeks [13]. Additionally, Ko et al. identified that the pancreatic □-cells in the untreated mice were severely damaged, while 75% of treated mice had perfectly healthy cells for about 6 weeks [12].

Table 1: Experimental Results Summary

Source	GOI	Outcome	Duration of results
Nan M., Park J., Myung C.	ADN	Diabetic mice that originally had very low ADN levels demonstrated 10-15 times higher ADN levels after treatment.	2 weeks
Kumar M., Hunag Y., Glinka Y., Prud'Homme G., Wang Q.	GLP-1/Fc	Researchers successfully treated mice with Type-2 diabetes with a plasmid vector to protect the GLP-1/Fc fusion protein from denaturing.	2 weeks
Jean M., Alameh M., Buschmann M., Merzouki A.	GLP-1	The treated diabetic mice had 5 times more GLP-1 in their bloodstream than untreated mice and insulin levels peaked at 3 times more than before.	3 weeks
Ko K., Lee M., Joon J., Wan S.	IL-4 and IL-10	Untreated mice had severely damaged pancreatic β-cells, while diabetes was prevented in about 75% of treated mice.	6 weeks
Martinenghi S., Cusella De Angelis G., Biressi S., Amadio S., Bifari F., Roncarolo M., Bordignon C., Falqui L.	Self-made GOI, ET method	Seventeen out of 20 diabetic mice expressed consistent levels of mature insulin after administration into the skeletal muscle.	6 weeks
Fukushima M., Hattori Y., Tsukada H., Koga K., Kajiwara E., Kawano K., Kobayashi T., Kamata K., Maitani Y.	ADN	At the end of the study, treated diabetic mice had significantly higher ADN levels than the untreated mice.	2 weeks

DISCUSSION:

Benefits of Longer Length

Based on observations from these gene therapeutic studies, the treatment effects have demonstrated remarkable consistency for 2, 3, and even up to 6 weeks in both types of diabetes [9, 11, 14, 10, 12, 13]. This suggests that individuals affected by Type-1 and Type-2 diabetes could anticipate significantly reduced frequency of injections compared to treatments of IIT requiring multiple daily injections or weekly injections for Ozempic. Gene therapy presents a

promising solution for diabetic patients experiencing cognitive, aging, or physical issues who struggle to cope with frequent injections.

Further Benefits of Plasmid Gene Therapy

The efficiency of plasmid gene therapy is likely attributed to the protective capabilities of plasmid vectors, particularly in the case of GLP-1 [10, 11]. Moreover, the security of plasmid vectors may indicate that concerns related to cross-interactions with other drugs, which can be problematic for treatments like Metformin, are not a hindrance during plasmid gene therapy [5].

Addressing Drawbacks

Plasmid gene therapy for diabetes is still at an experimental stage and further examinations of its safety should be addressed. Still, the promising results that this unique treatment has to offer show the potential of it to eclipse current treatments. The ability to treat both forms of diabetes simply by changing the GOI has huge potential in cost-effective research, which is why advancement to human test subjects is necessary. The many similarities were salient to the synthesis of the key points of these experiments, but their differences in the type of diabetes, the GOIs, etc are important to help understand the versatility and effectiveness that plasmids ultimately hold in biomedicine.

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