

ARE SYNTHETICALLY PRODUCED siRNAs CAPABLE
OF COMBATING TYPE I DIABETES MELLITUS IN DOGS?

BY

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Abstract:

The RNA interference pathway pre-exists in cellular functions to protect the cell against viral control. Short pieces of RNA (which code for a gene) can be inserted into cells by transfection to silence a specific gene. This paper proposes ways of using small interfering RNA (siRNA) strands to combat type I diabetes mellitus, an autoimmune disease, in canines.

The approach suggested is the targeting of B lymphocytes with the correct siRNA sequence to prevent antibodies being produced, which attack and destroy β cells in the Islets of Langerhans. This means that the dog can still produce insulin and control its own blood sugar levels. However there is a risk of triggering interferon responses and off-setting of genes when using siRNAs, which needs to be considered. Additionally the economic and commercial viability of batch processing and manufacturing unique siRNAs is discussed.

Introduction:

Recently there has been a major scientific breakthrough with the discovery of small interfering RNAs (siRNAs), which play an important part in the RNA interference (RNAi) pathway. In 2006 Andrew Fire and Craig Mello were awarded the Nobel Prize in medicine for their discovery of this process [1]. Their aim was to understand gene expression and they found that when double stranded RNA (dsRNA), which coded for a particular gene was introduced, that gene was silenced. With further research into the process they found that there is already a naturally occurring pathway. When dsRNA is introduced into an animal cell, for example by a virus, a protein enzyme called dicer cuts the dsRNA into smaller pieces of about 21 nucleotides long. These are called siRNAs, which are essential for turning off genes. They have an overhang of 2 nucleotides at each end, which allows them to be 'recognised' by RISC (RNA Induced Silencing Complex). RISC separates the dsRNA; one strand is discarded and the other is complementary to the messenger RNA (mRNA), which is produced from transcription of the uncoiled section of DNA. RISC then brings the strand of siRNA to the mRNA and they form a double strand. This attachment inactivates the mRNA and induces cleavage of mRNA; by degrading the transcribed mRNA from a gene it prevents proteins being produced from that gene. As long as the siRNA has the same base sequence as the DNA this was translated, then we can silence, or 'turn off' that gene. The entire base sequence is known for the Human Genome, however the functions of some of these genes are not known. By silencing one gene at a time their function is revealed. Research is underway into synthetically manufacturing specific siRNAs. This new easy technique for silencing genes is being used in medical research to cure diseases such as cancer, autoimmune diseases, dominant genetic disorders and viral infections. It is also relevant to veterinary medicine as there are many zoonotic viral diseases such as avian flu. An example of a successful veterinary trial that has already been carried out was on monkeys to lower their cholesterol.

For further reading see Biological Sciences review, Volume 22 September 2009 edition, 'Small RNA, big impact' article by Ray O'Keefe.

Discussion:

So far the research into the uses of siRNAs has focused on discovering gene functions and preventing a number of diseases. This paper considers how siRNAs could be used to treat type I diabetes mellitus in dogs.

Type I diabetes mellitus is an autoimmune disease where the immune system attacks self cells. It is also an endocrine disease [2], which affects the pancreas and prevents it producing insulin. This occurs when antibodies destroy the insulin producing β cells in the Islets of Langerhans [3]. This means that the affected dog can no longer control its blood glucose levels. In canines, 1 out of 500 dogs will develop diabetes [4] and 99% [5] of these will develop type I diabetes mellitus. Typical signs of diabetes in dogs are excess food and water consumption, increased urination, dehydration and weight loss [6]. Dogs can also develop cataracts [6]. Diabetes can become very serious if left untreated because it causes the syndrome ketoacidosis which happens when the body 'thinks' it is starving and starts to break down fatty acids and the liver produces organic acids called ketones [7] – these give the characteristic sweet smelling 'pear-drop' breath. The current most effective treatment for this condition is insulin injections every 12 hours and after a meal [7].

From this knowledge of diabetes I have identified two hypotheses for why the β cells get attacked in a diabetic dog:

- 1) The antibody is different
- 2) The antigen is different

First Hypothesis:

This option looks at how diabetes could be caused through a mutation within the B cells (white blood cells which produce antibodies and mature in the bone marrow [3]), which are producing antibodies that attack self cells. On the outside of every self cell there is the same specific antigen, which allows it to be recognised as a self cell to prevent it from being attacked by the body. However if a mutation did occur in the B cells, which caused them to have an antigen-binding site complementary to the antigen on the surface of the self cells, then they would produce antibodies to attack the body cells.

To find the particular antibody, which is attacking the β cells in diabetic dogs, a blood sample could be taken to find it. Once found, the DNA base sequence, which codes for the gene, which makes up the antibody needs to be translated. Particularly for the antigen-binding site as this is the variable region. Antibodies are made up of four protein strands [8], however if just one of the genes that codes for a protein within the antibody is silenced then it cannot function. To find the antibody a comparison between blood samples of a non-diabetic and diabetic dog might be needed to deduce the incorrect antibody because non-diabetic dogs will not produce it.

Second hypothesis:

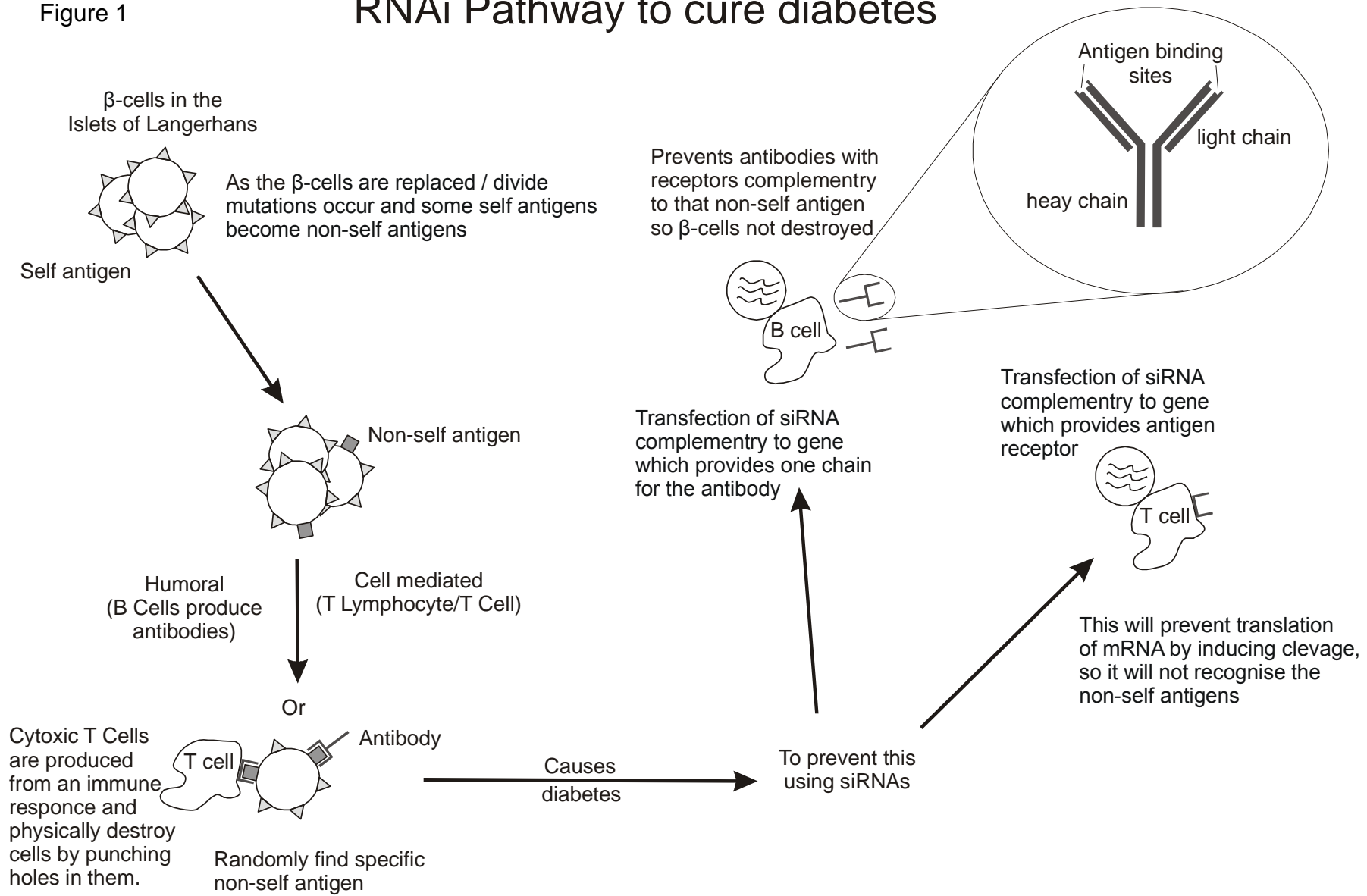
A more likely hypothesis is that the mutation is occurring in the coding process for the 'self' antigens that are expressed on the surface of the β cells meaning the antigen would become 'non-self'. This could cause an immune response, because the antigen would be seen as a foreign protein and T cells (T Lymphocytes are types of white blood cells which mature in the Thymus) [3] attack these cells because they are seen as antigen presenting cells. The T cells stimulate B lymphocytes to be produced with complementary antibodies to the non-self antigens of the β cells. Once the antibodies have attacked the β cells, their function is immobilised so no insulin can be produced. It is therefore these B cells that need to be targeted with siRNAs.

Using siRNAs to combat diabetes

Either way the best method would be to switch off the production of antibodies by inhibiting the gene within the B cells (see figure 1). This is because one cannot be sure that the antigen is a protein, rather than a glycoprotein or polysaccharide [8]. Also it is much easier to take a blood sample to find the incorrect antibodies rather than taking β cells from the pancreas to find the incorrect antigen. Once the base sequence for the DNA of the gene that needs to be silenced within the B cell has been found, then the RNAi process can begin. Companies have already been set up to artificially manufacture siRNAs such as Sirna Therapeutics [13]. This method produces many single short strands of RNA with the correct codons so that it is complementary to the mRNA, which needs to be cleaved. With the correct siRNAs produced, they can be introduced into small lipid spheres where they are contained. To make sure that all B Cells with the mutation receive the siRNAs they could be injected into the blood, because B Cells are part of the humoral response [3]. These lipid spheres can travel round the body and will be accepted by any cell with a cell membrane through transfection, which is a way of introducing fairly large molecules into cells [14]. This works because the cell membrane is a phospholipid bilayer [8], which will allow the lipid sphere to bind with it to form a vesicle and then release its contents into the cell. Once the siRNA is inside the cell the protein complex RISC will bring it together with the mRNA which has been transcribed so that they can bind together and induce cleavage of the mRNA. This means that the protein is not produced and so the β cells are no longer attacked.

Figure 1

RNAi Pathway to cure diabetes



Conclusion:

Overall one can conclude that siRNAs could theoretically be used to treat diabetes successfully.

The first hypothesis is unlikely because if the antibodies are attacking self cells, why is it concentrated on the β cells. It also seems unlikely that so many dogs get a mutation in the β cells, which means they produce complementary antibodies to their own self antigens. However the second hypothesis is more likely because it is only the β cells, which are being targeted. It seems more probable that a non-self antigen is being made by the cells, so that they are seen as foreign and get attacked by antibodies.

Using siRNAs to treat this auto immune disease has advantages over using insulin. The dog still produces its own insulin and can eat a normal diet, as it will be able to regulate its own blood sugar, so no injections are needed after meals. siRNAs are not passed on through cell division, so depending on how often β cells divide; injections might not be needed every day.

On the other hand, this approach is not economically viable. Normally dog owners give the insulin injection in the scruff of the neck or flank [15]. However with using siRNAs the injection would be into the blood. This means that a vet has to do the injection, which will be expensive. Also the mutation of the antigens present on the surface of the β cells are different in each dog, so they will all require unique siRNA sequences. siRNAs are artificially produced and will be expensive for companies to make using a batch process, however the president of Sirna, Howard Robin suggest that they will soon be able to manufacture them at a reasonable price [13].

There is a risk of triggering an interferon response when using siRNAs, which is a generalised non-specific response to viral infections. It is likely that it occurs due to the introduction of foreign DNA/RNA and therefore could occur with artificially produced RNA. According to scientific research, there may be a link between interferon responses being triggered and the length of the siRNAs. If the siRNA is less than 30 nucleotides long then a response is unlikely to happen [11].

Another consideration is off-setting, which could be a major problem, when siRNAs are produced artificially. There is a 10% chance that they will target a different gene [9] and this could lead to unwanted toxicities [10]. Even if the siRNAs are a perfect match to the mRNA they can still bind to other mRNA strands forming imperfect matches, for example G:U wobbles, mismatches and bulges [11]. These effects compromise the specificity of RNAi if the sequence identity between siRNAs and random mRNA transcripts causes RNAi to prevent non-targeted genes from functioning [9]. Scientists have found that high-sequence specificity and low probability for off-target reactivity were optimally balanced at 21 nucleotides in length. The chance of off-setting increased (not always significantly) with greater chain length of dsRNA [9].

Overall from the research it is possible to say that siRNAs could be an effective treatment for diabetes in canines. However the main problems that need resolving for the treatment to become viable are making the batch process more efficient and less expensive as well as creating an appropriate delivery mechanism.

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