

How to deliver the siRNA complexes
to all cells requiring them?

BY

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PASS

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ABSTRACT

In this paper I aim to discuss how it would be possible to deliver siRNA to those cells that need them directly. I have learnt about RNA within my biology lessons in school, however at the vet-medlink conference I learnt about the new technology of using siRNA in order to stop the mRNA reaching the ribosome's and producing certain proteins. The central idea to my work is the possibility of using different delivery methods for the siRNA into our cells, especially the concept of using stem cells to do this. Naturally I will consider the ethical side of the delivery.

INTRODUCTION

Recently there has been development in RNA Interference, RNAi is a technology which can control which genes within living cells are active, and how active they are. The technology would also allow us to control the genes by switching them off. The research has found that if we were able to find a way in which we could deliver the RNAi directly into specific cells, we would be able to stop the development of cancer cells, HIV, Foot and Mouth or even Tuberculosis for example. This is very important to veterinary medicine as it would mean that we could prevent the development of the disease within an animal and thus theoretically curing it without putting them through more pain. If it were possible then to screen the animals before the development of the disease for the cells or pathogens which cause the disease we might even be able to prevent them from even developing the disease in the first place. Not only would this be better for the animal, but it would be better for the owner as they would not have to go through the pain and stress of seeing their beloved pet ill or even in pain from a disease that with the RNAi would be able to prevent. The prevention of an animal becoming ill is the main aim of a vet, and they would rather much be preventing the disease from happening in the first place than having to treat it afterwards, and face the fact that it maybe incurable.

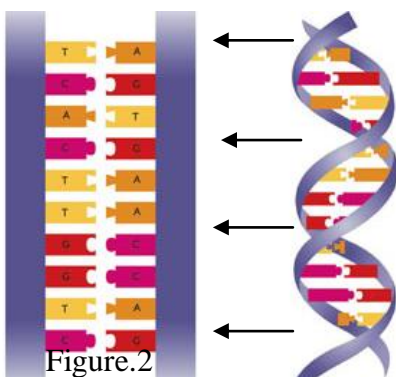
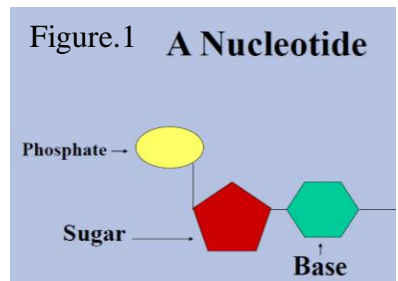
Before I go further in introducing that of which I am going to discuss, I thought that I should explain the basics of RNAi. First of all I should explain that RNAi stands for Ribose Nucleic Acid interference. RNA is single stranded and is found within our cells. RNA is made out of pentose sugar, a group of phosphates and a base – Adenine, Cytosine, Guanine or Uracil (figure 1). DNA is made out of exactly the same molecules as RNA, other than Thymine is present instead of Uracil within the bases.

As you would imagine DNA is very specific and only certain bases will bond with each other. The bases can be divided into two types, purines (Adenine and Guanine) and pyrimidines (Cytosine and

Thymine/Uracil). It is only possible for one from each type to bond with another, even more they are only able to bond in certain pairs; Adenine will always bond with Thymine (Uracil), while Guanine will always bond with Cytosine.

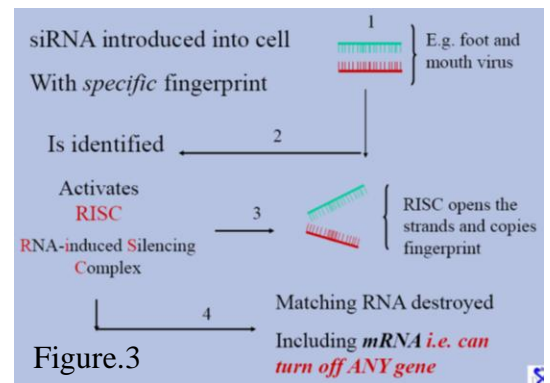
To be able to understand how RNAi would work, you first need to understand how proteins are made. First DNA has to be transcribed and then translated before it can become protein. During the phase of transcription the DNA is unfolded from its helix (figure 2.). During the process of RNA polymerase, an individual strand and the bases are copied along with their instructions, which include what they should build and breakdown. This is what forms the messengerRNA which are sent out of

the nucleus into the cell, these then go to the ribosome's where transcription then happens. This is the process in which mRNA reaches the ribosome and then proteins are produced. The idea behind RNAi is that the mRNA would never reach the ribosome, thus the process of translation would never happen. This literally would mean that we could stop genes from being switched on, or even we could just switch them



off at any time, we feel that we need to do so. If this would be possible to do we could switch off cancer cells, create a cure for foot and mouth.

Unlike plants and invertebrates, humans and animals (vertebrates) do not use the viral defence. This is very important as this allows us to use siRNA as a treatment. Otherwise if this defence was used, the RISC (RNA-Induced Silencing Complex) would open up and copy the 'fingerprint' strand of the dsRNA, which has invaded the cells, and has already been broken up by the Dicer enzyme. Once RISC has been activated the matching RNA is destroyed (figure 3.), yet the cells will always remember the virus which has invaded the cell, and will employ this reaction quicker. Even though Dicer and RISC are present in the cells of the higher animals, the presence of the long chain of dsRNA creates a complex inflammatory response of the interferon, which shuts down the production of all proteins. Fortunately the RNAi pathways are present within human cells. This means that specific cells will be able to learn to destroy specific genetic fingerprints which are produced by foreign RNA that have entered the cell. The most important aspect of this is the fact that they would also be able to destroy our own mRNA, which would then be able to stop the production of certain proteins within the cells. However because within human cells there are long strands of dsRNA present, the RNAi pathways have no effect on the outcome. This is due to the fact that because of their presence it triggers an interferon response which shuts down everything.



However in 2006 The Nobel Prize winner in Physiology and Medicine found that the introduction of a short double strand of RNA (siRNA) into the cells did not trigger the inflammatory response, but it does trigger an interference response instead. These siRNA would have to be no longer than 23 nucleotide pairs in length. Fire and Mello of Stanford School of Medicine first found this unique response in 1998, which they then published an article on it in Nature, which detailed the RNAi response within worms. The reason why this discovery won the Nobel Prize was because of its revolutionary affect in medicine. The reason for this revolution is the fact that it will go through the same procedures as the RNA would normally (figure 3), however when siRNA is entered into the cell and the matching RNA is destroyed, it also destroys the mRNA. This basically means that theoretically we would be able to switch off any gene. If you consider this it would enable us to prevent practically any virus, turn of detrimental cells such as cancer, Alzheimer's in humans, and perhaps even stopping autoimmune reactions, thus stopping the development of diabetes. This would even perhaps allow veterinarians to try and prevent the hereditary disease which are more common within pure breed animals, for example seizures in dogs. Recent research has found that RNAi has the potential to become a major technology that has therapeutic applications. Due to the use of carcinogenesis, it has created the opportunity in researching in to the use of the RNAi technology to target those molecules in the pathways, such at tumours which you would otherwise normally be untreatable, as they are resistant to chemotherapy or radiotherapy. This is beneficial to the veterinary medicine field as it would mean instead of multiple treatment of a tumour which would not cure it, but would cause a lot of pain, instead of this it could possibly mean fewer treatments which put the animal through less pain. The recent research has found that this has a significant effect on the antiproliferation (prevention of the spread of cells, e.g. stopping malignant cells from spreading into the surrounding healthy tissue), as demonstrated by the research in the December issue on nature.com (Wu et al., 2005).

DISCUSSION

For the following discussion in the delivery of the siRNA into the cells I will use the treatment of leukaemia, whilst I discuss the different possibilities in doing this. At the moment there are many different delivery technologies which are available at the moment to deliver the siRNA into the cells. Some of these technologies are ones such as compounding the siRNA with cationic lipids, thus making the siRNA more positively charged. The fact that it is encased within a lipid means that it would be able to move through the cell membrane through diffusion. Other techniques comprise of encapsulation the siRNA in a liposome, condensing polymers, or joining the siRNA with antibodies. Even with these techniques available it is still difficult to deliver the molecules of siRNA into a mammalian cell effectively. However after doing some research I have noticed that the most promising delivery of siRNA into the cells would be to join the siRNA with cell penetrating peptides. This technique has resulted in cytotoxicity which is associated with the limited delivery of the siRNA into the target cells, as demonstrated Dowdy et al., (2008), "Enhancing the cellular uptake of siRNA duplexes following non-covalent packing with protein transduction domain peptides".

Even though I believe this is a very promising technique, I would consider saying that personally I would believe that if it were possible to join the siRNA with the stem cells of the individual needing treatment, I would think to be a better option. The use of the stem cell technology which is fairly modern also would allow use theoretically to deliver the siRNA to the specific cells without triggering an immune system response. Theoretically the reason why this would not happen is because the cells are taken directly from those who are suffering from a disease or illness. There are two different types of stem cells that are available to use, the first being embryonic stem cells and the other being adult stem cells. Considering that this technology comes with its objectors along with those who are deeply in favour of it. Due to this I will consider the ethical side of the use of this treatment.

The use of embryonic stem cells in order to deliver the siRNA into our cells, in order to treat leukaemia would have more of an advantage over adult stem cells as they have a greater number of different types of differentiated cell types which they can become. The reason for this is that they are pluripotent, while adult stem cells are not. In order to introduce the siRNA into any of the stem cells they would have to be taken, and then treated in vitro, as this would not normally occur within the human body. This would cost the owner of the animal or the human I would imagine a bit of money as it would be fairly costly. However if you consider that if this treatment were to be successful in treating a disease such as Leukaemia, it would mean that the patient would no longer need drugs in order to help them combat the illness. This would be a welcome message to those suffering from the disease as the drugs such as prednisone, vincristine and daunorubicin do not cure the disease; they simply just prolong the patient's life (names of drugs are from the WebMed website). If treatment through using stem cells in order to deliver the siRNA into the cells which cause the disease was successful, it would mean that they would not have to worry about forgetting to take their drugs, as the siRNA would have stopped the production of the proteins which cause Leukaemia, thus meaning that they would be cured from the disease. Also another benefit in which using stem cells has is that it has more of an ability to find specific cells in order to treat them, and then the fact that they could be used to repair the tissue that was damaged by the disease. However these would more than likely have to be two different samples of stem cells, as they would be treated in the lab to do two totally different tasks. The reason that they would be able to do multiple tasks simply

boils down to the fact that they are undifferentiated cells, which means that theoretically we could change them into any cell that we want, as long as we know the means to do this.

In order to do this however, you would have to find a way in which you could combine the siRNA with the stem cells before you could even think about how to deliver them into the body. Theoretically you should be able to use the technology in which you change the stem cells from being undifferentiating cells into specific cells to do this. During this process you would just have to add another step into it in order to join them with the siRNA. Just going on the information I have learnt from my studies I believe that it may be possible to join the siRNA to the stem cells through using an electric current. This would charge both the siRNA and the stem cells, with the hope then that due to these charges they would attract each other, and thus forming a bond that would hold them together. However after considering this, I have doubt to whether this would be effective, as it has possibly a chance of activating the immune system, which would possibly see it as something foreign to the patient's body.

Therefore I have considered the possibility of using the same process which is used to fertilise eggs during IVF. Through removing and destroying the genetic information which is held within the cells, this would leave us with a cell which we would be able to inject the siRNA into. I believe that this has a higher chance of being successful as it would mean that the outer part of the cell – its membrane would still be totally the same genetically as the patients DNA. While inside the membrane in its cytoplasm would be the siRNA, which would have taken the place of the original DNA. This would mean that there would be a lower chance of the cells being attacked by the immune system when they are delivered into the body. This is a key factor when considering different methods of delivery of the siRNA, especially looking at if the delivery would have any side effect.

Once you have successfully managed to combine the siRNA and the stem cells, you are faced with the task of introducing it into the patient's body. The most obvious way of doing this would be to do it through a form of injection, which would not be too hard to do. The hard part of this would be to create the easiest way in which that the cells could actually be injected. I believe that this could be done through putting the cells in a liquid like solution which could simply be injected into the body, and the carried to the specific place where it is needed through the blood stream. A very important thing to consider when thinking about using stem cells for the delivery of the siRNA is what stem cells should be used. There is a very strong ethical debate about the use of embryonic stem cells. With embryonic stem cells it can depend of a person's interpretation of when life begins, whether it's at fertilisation, 20 weeks, birth? Nobody is able to define a line in when the embryo becomes a foetus with the potential for becoming a human being/life or when it is still just cells. On the one side many believe that an embryo is created to save a person's life that it is defying nature and going against 'gods will'. This idea tends to now lead on to the thought that making parents and people who are unethical and gives them the belief that it is acceptable to create life and take it away at our whim. Thus creating a generation of people who are unethical and take things for granted.

Others believe that through the use of embryonic stem cells we are destroying the potential for life, especially as we are creating a life and then taking it away from them before they even have the chance to live it. Some people also have an issue with the fact that the embryo is created specifically for stem cells, and the fact that there may be a chance of the embryo still living after its cells have been used. This happens when the embryo is allowed to develop fully into a baby and then they use the umbilical cord,

and then the baby itself can also become a secondary donor through their bone marrow, this was portrayed recently through the film 'My Sister's Keeper'. The process of donating could then also really have bad side effects, e.g. shorter life, infertility, disabilities, especially if the donating happens at an early age. Although there tends to be more ethical belief against embryonic cells, others take the view point that they can be used to stop suffering and diseases, and that this overrules the other issues. While others have the belief that it is better to do it as it helps a living person.

However there seems to be fewer objections against the use of adult stem cells, as these do not have the same ramifications. The reason for this is simply because it does not involve the use of embryos. Adult stem cells can be cultivated through collecting some of the patient's bone marrow or even fat, and extracting the cells from these. There tends to be few ethical issues with these stem cells, as long as the person gives consent. Although this can be different when it is a child, as they are unable to give consent, as it can cause problems, especially in the young. One thing to take into consideration is the fact that overpopulation is becoming a bigger issue, and that in the near future it will be very easy for people to use embryonic and adult stem cells in order to preserve life. What we have to consider is the question that is the use of stem cells to preserve life against the laws of nature? And also we have to consider the fact that the presence of disease could be a natural way of nature trying to keep control of the numbers there are. The fact is that if we are successful in solving the use of stem cells to prevent disease, we are ultimately going to have fewer deaths each year, which will mean that overpopulation would become a bigger issue.

CONCLUSION

Overall after looking into the different delivery methods that there are available now, and considering the concepts I have in order of delivering the siRNA into the body, I fully believe that within the next few decades or so, this technology will be frequently used in the treatments of diseases, first in humans, then making its way in to the veterinary field. In summary I believe that joint use of stem cells being either one and that of siRNA would be a very effective way in curing the disease that a lot of patients suffer from today. This would also bring a way in which stem cell research can be further developed, which could in the future then lead on to further developments within the two fields, or even beyond. Although my ideas have brought up the fact that this form of treatment could be fairly costly, it is likely that as the treatment becomes more common, the cost will slowly begin to drop. However it is unlikely that it would become something that the majority of people could afford to do without having to sacrifice something in the first place. You could put it simply, that if you truly care about someone you would normally be more than willing to sacrifice something or work for it, if it meant that those you loved could be cured.

If there was to be any problem with my idea it would be the way in which the combined siRNA and stem cells would be injected into the body. I am sure that there are several ways in which this would be possible in doing, however it would have to be done in a high-tech laboratory, which could mean that things would have to be sent away to do so. Also as this would be very specialist, which would initially the cost to do so could be rather high. It could take a long time in perfecting the solution, which would mean a lot of research, time and most importantly money. However maybe in the future, some research might develop a concept in which it would lend itself perfectly to this treatment, in the way RNAi did to stem cell. Also as technology develops it will become easier to overcome such problems, and it would likely happen much quicker.

REFERENCES

1. Haoquan Wu, Sang-Soo Kim, Chunting Ye, Priti Kumar, Isaac Chiu, Sandesh Subramanya, Premalata Shankar and N Manjunath, published an article on the Molecular Therapy section on Nature.com in December 2005. The article was detailing the Target Delivery of siRNA to Macrophage for Anti-inflammatory Treatment.

<http://www.nature.com/mt/journal/vaop/ncurrent/full/mt201027a.html>

2. Andrew Fire and Craig Mello's work on the discovery RNAi mechanism, won them the Nobel Prize for Physiology or Medicine in 2006.

http://nobelprize.org/nobel_prizes/medicine/laureates/2006/adv.html

3. Steven Dowdy and B.R. Meade published an article on "Enhancing the cellular uptake of siRNA duplexes following non-covalent packing with protein transduction domain peptides". The article was published on the PubMed.gov website which is a part of the NCBI website in October 2007.

<http://www.ncbi.nlm.nih.gov/pubmed/18155315>

4. An example of some of the drugs which are used to treat one type of Leukaemia came from the WebMed website. The drugs I listed are used to treat Acute Lymphoblast Leukaemia (ALL).

<http://www.webmd.com/cancer/tc/leukemia-medications>