

THE TREATMENT OF BLUETONGUE DISEASE USING RNA  
INTERFERENCE AND POSSIBLE METHODS OF INSERTING IT  
INTO INFECTED CELLS

BY

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**PASS WITH MERIT**

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## ABSTRACT

The principle of RNA interference was first discovered in the early 1990's when researchers attempted to alter the colour of petunias to a deeper purple. The unexpected result was found to be caused by post-transcriptional inhibition of gene expression caused by increased mRNA degradation. Following the discovery researchers around the world searched for similar observations in other living organisms. Craig Mello and Andrew Fire's 1998 report showed evidence for gene silencing after injecting double stranded RNA into *Caenorhabditis elegans*. Since then research has focused on using RNA in treatments for disease. This report explores the methods of introducing RNA into animal cells without causing the inflammatory response and in particular, the possibility of RNA being used in the treatment of Bluetongue disease.

## INTRODUCTION

The principle of RNA interference was discovered in the early 1990's when researchers in the US and the Netherlands attempted to alter the colour of petunias to a deeper purple. Investigations indicated that the unexpected outcome (after adding the gene encoding for the specific pigmentation) was due to post-transcriptional inhibition of gene expression caused by the increased rate of mRNA degradation. This effect was named co-suppression. At the time it was unknown what caused the effect in the pigmentation. [http://wikipedia.org/wiki/RNA\\_interference](http://wikipedia.org/wiki/RNA_interference)

After the discovery in plants, researchers around the world searched for similar observations in other living organisms. Craig Mello and Andrew Fire's 1998 report showed evidence for gene silencing after injecting double stranded RNA into *Caenorhabditis elegans*. They investigated the regulation of muscle protein and the effects of injecting the *C. elegans* with mRNA, antisense RNA and double stranded RNA. They noted that only the double stranded RNA successfully silenced the targeted gene therefore calling the process RNAi. In 2006 Fire and Mello were awarded the Nobel Prize in physiology or medicine for their work. [http://wikipedia.org/wiki/RNA\\_interference](http://wikipedia.org/wiki/RNA_interference)

RNA interference (RNAi) is a post-transcription process whereby double stranded RNA can be inserted into a cell and the target gene becomes 'switched off' or silenced. There are two types of RNA molecules essential for this process, micro RNA (miRNA) and small interfering RNA (siRNA). RNAs are the product of genes, they can bind to other specific RNAs and increase or decrease the activity. Before translation occurs protein synthesis is prevented by selective degradation of its encoded messenger RNA (mRNA)(Milhavet et al, 2003).

This process is used in plants and invertebrates as protection against viruses. The RNA pathway is found in other eukaryotes. However in plants there are more variations of the enzyme Dicer, which cleaves long double stranded RNA into short fragments of up to 23 nucleotides. [http://wikipedia.org/wiki/RNA\\_interference](http://wikipedia.org/wiki/RNA_interference)

In humans the presence of long chains of double stranded (dsRNA) causes an interferon based inflammatory response which stops protein synthesis which would therefore prevent the RISC complex from being formed. If small interfering double strands of RNA (siRNA) of no longer than 23 nucleotide pairs long are introduced into cells it does not trigger the inflammatory response, it triggers the interferon response.

The current research into RNAi focuses on how to overcome the problem of how RNA can be used in the future in medical treatments for both humans and animals to protect against parasitic genes and viruses including hepatitis C virus (HCV) and human immunodeficiency virus (HIV). It could also be used as a possible treatment for other diseases such as cancer.

Currently RNAi is mainly used in cell culture and vivo studies (experimentation using a whole organism) aimed at understanding the function of a particular protein. By studying the effects of the decrease in activity of the targeted gene it can be determined what role the gene plays in cellular activity.

There are however some difficulties with using RNAi to cure or treat illnesses as it is difficult to insert into every cell that requires it. Another problem is that the long strands of RNA cause the inflammatory response; therefore siRNA would need to be implanted as it does not produce this response. There are problems however with using the shorter more specific RNA because it can have 'off target' effects. These effects occur when the RNA that is introduced has a base pair sequence that can bind with more than one gene.

This report will focus on how siRNA's can be inserted into cells and the possible treatment of Bluetongue disease using this process.

## DISCUSSION

RNA interference has the potential to cure or treat a variety of diseases in future generations. In particular RNAi could be widely used in Gene Therapy to treat hereditary conditions such as severe combined immunodeficiency disease (SCID's) and cystic fibrosis. Other proposed medical uses of RNAi include use as a treatment for cancers by silencing the genes which are involved in cell division, to prevent HIV from spreading. It could also be used to silence influenza gene expression (although these

would be more complex due to the different strains of the virus and its adaptability). There is also the potential for RNAi to be used as a treatment for neurodegenerative diseases such as Huntington's disease and Alzheimer's. However there are complications as there are many different proteins which result in the degeneration.

There could also be developments in veterinary medicine treatments for diseases such as Foot and Mouth and Bluetongue Disease. This report will focus on the possibility of treating Bluetongue disease using RNAi and possible ways of inserting it into the specific cells.

Bluetongue or catarrhal fever is a non contagious, insect - borne viral disease which affects sheep, cattle and other ruminants. Bluetongue is caused by the pathogenic virus, Bluetongue virus (BTV) of the genus orbivirus, a member of the Reoviridae family. There are 24 serotypes. [http://en.wikipedia.org/wiki/Bluetongue\\_disease](http://en.wikipedia.org/wiki/Bluetongue_disease) A serotype is a group of closely related microorganisms classified by a similar set of antigens. [www.asgt.org/about\\_gene\\_therapy/terminology.php](http://www.asgt.org/about_gene_therapy/terminology.php) The virus is transmitted by a vector, similar to the mosquito that causes Malaria in humans. They are known as *Culicoides imicola* as well as other culicoids.

Bluetongue disease causes severe illness for the animals it infects. At present there is no cure for the disease. Animals can however be vaccinated with a mild form of the version which is most prevalent that season. There is no way of controlling where the disease strikes as it relies on the weather, which determines whether the midge will be located in certain areas. Control measures include setting up protection and surveillance zones around an outbreak, enforcing movement restrictions and, if appropriate, slaughter and/or vaccination of the animals involved. Even if the animal is vaccinated with BTV-8 for example this vaccine does not offer cross protection against any of the other 23 serotypes. Other preventative measures can be taken including vector control through the use of ectoparasiticides, housing ruminants during peak vector activity and practising good biosecurity, although these are not as effective.

[http://www.bva.co.uk/activity\\_and\\_advice/1108.aspx](http://www.bva.co.uk/activity_and_advice/1108.aspx)

Research into Bluetongue disease has found that with climate change the virus can increase its range by establishing itself in other vector species. Researchers have also looked at how climate change affects transmission. They have found that the virus can remain undetected within host immune cells, which can explain why the virus can suddenly appear in a geographical area. If there is a high likelihood of the virus being more prevalent in the future, the virus will cause more problems for farmers. However if a treatment was found increased cases would no longer be a problem as instead of paying for the vaccine or losing stock if the animals are culled the farmer would only

have to pay for treatment if his animals became infected.

[http://www.iah.bbsrc.ac.uk/disease/bt\\_aw.shtml](http://www.iah.bbsrc.ac.uk/disease/bt_aw.shtml)

Treatment with RNA interference technology would mean that the vaccine would no longer be necessary as the treatment would be used for all serotypes as they would have a similar genetic makeup. The treatment would mean that the animals infected with the virus would be given the specific siRNA that would code for the gene that causes the problem. One of the two strands (the guide strand) of siRNA would be incorporated into the RNA-induced silencing complex (RISC). When the guide strand base pairs with a complementary sequence of messenger RNA (mRNA) it induces cleavage by Argonaute, the catalytic component of the RISC complex. This means that the virus is unable to reproduce and therefore cause the symptoms associated with Bluetongue. [http://en.wikipedia.org/wiki/RNA\\_interference](http://en.wikipedia.org/wiki/RNA_interference)

For this treatment to effectively work and destroy the virus there has to be an effective technique of administering the siRNA's into the cells that require it. There are many different methods that could be used including some used for gene therapy such as using a virus as a vector, using a liposome or using a plasmid. Other methods could include using an agrobacterium or a gene gun. These methods are detailed below in paragraphs 1-5.

1) Plasmids (shown in figure 1) have been used to try to treat congenital muscular dystrophy but have encountered problems in that they can cause damage to muscle.

<http://www.nature.com/gt/journal/v10/n6/full/3301927a.html>

The principle involved is that the plasmid is cleaved using a restriction enzyme and the specific gene can be inserted into the plasmid. The plasmid is then rejoined together using the same enzyme.

<http://library.thinkquest.org/C004367/be9.shtml> Plasmids have also been used to replicate insulin. This process has been much more effective as the plasmids are not inserted into the human. The insulin produced can be filtered and used to treat diabetic people. (Sue Hocking, Pete Kennedy *et al.*)

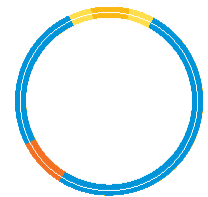


Figure 1

The problems associated with using this method is that the plasmid method is used for inserting DNA. This had not been tried using RNA. This would mean that the RNA wouldn't be incorporated in the plasmid. This method has also only been effective with reference to the production of insulin and some other proteins but only in bacteria. This method has also not been very successful when used in trials on humans at present; therefore farmers would not be willing to pay for treatment which wouldn't be

effective in curing their animals as this method would not be able to introduce RNA into the cow's cells.

2) *Agrobacterium tumefaciens* is regarded as a useful gene delivery system as it is able to carry any gene within the T-complex. That specific gene can be inserted into the target plant's DNA with a high degree of success. This method is so effective because unlike other mobile genetic elements such as retroviruses, the T-DNA strand does not encode for other functions required for movement and integration of the DNA. The gene of interest will automatically be inserted into the host plant's nucleus, (replacing the T- DNA strand), with a high degree of success with relatively little human intervention. <http://arabidopsis.info/students/agrobacterium/uses.html>

Again there are problems with this method as it can only be used in plants and again it uses DNA not RNA and therefore there is no possibility of this method being used in the near future as there are too many problems to overcome to make it worthwhile.

3) A gene gun could be used as a way of inserting the RNA. Under certain conditions the RNA can become sticky adhering to biologically inert particles such as metal atoms such as gold. By accelerating the RNA particle in a partial vacuum and introducing the target tissue within the path, the RNA would be introduced. This method has only been used in cell culture and the cells that take up the desired RNA are then cloned. Obviously this method could not be used on a whole animal as it would cause damage to healthy cells in the animal. There is also no way of telling how many cells contain the siRNA's, meaning that the treatment would only occur in a localised area. [www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/McDonaldGe](http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/McDonaldGe)

4) A virus could also be used as a vector. Instead of inserting the virus's genetic material which would cause disease, RNA could be inserted. Retroviruses would be the most effective virus to use for inserting RNA. [http://en.wikipedia.org/wiki/Gene\\_therapy](http://en.wikipedia.org/wiki/Gene_therapy)

The virus would be an accurate way of inserting the gene and it would affect many cells not just the one it is inserted into. However there are many issues related to using a virus. These include the fact that Bluetongue disease itself is caused by a virus and there may be some scepticism over whether treating a virus with a virus will be effective. There is also a chance the virus could mutate and regain its ability to cause disease.

In previous cases, when trialled to treat humans with severe combined immunodeficiency disease, (SCIDS), it lead to the children developing a leukaemia like condition and some patients died. Because of this there may be some opposition of this treatment being used, particularly to treat human diseases. There is also a short-term

cure with this method as the rapidly dividing nature of the cells may prevent the treatment from achieving long term effects which means that patients would have to undergo multiple treatments. In a farm situation this would not be preferable as farming is a business and therefore farmers do not have the resources to spend on repeated treatments. It may be difficult to repeat treatments using viruses as the animal's immune system would just destroy the virus if the treatment was repeated.

[http://www.ornl.gov/sci/techresources/Human\\_Genome/medicine/genetherapy.shtml](http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml)

The final method that could be used to insert the siRNA's into cells is the use of a liposome. As the liposomes are made of phospholipids they can pass directly through membranes and enter cells by endocytosis. This method is not toxic to the patient and the lipoplexes are able to carry more DNA and therefore more RNA than viral vectors, (Miller 1997). The problem associated with this method is that it is currently used for DNA not RNA but as these are very closely linked it is likely that it could work using RNA. There are also problems with targeting the cells which require the treatment.

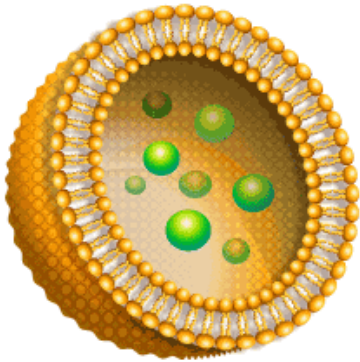


Figure 2

Cationic lipids can potentially be targeted to specific cell types by incorporating certain ligands into their structure. These ligands would bind to cell-surface receptors uniquely expressed on the target cells and would help ensure that the therapeutic RNA finds the disease-causing cells. Incorporating such ligands into cationic liposomes is an area of high research interest. At the moment there has not been very much research into the uses of liposomes in treating diseases and therefore this may not be very useful in the coming years but may be of more interest to future generations.

## CONCLUSION

Overall there are many problems that would need to be overcome before siRNA could be used as a treatment for a specific disease. A general problem is that the off target effects would need to be overcome to ensure that the siRNA is only specific to the gene which requires silencing and not to an actual piece of mRNA that encodes for an important protein that is needed by the animal or human. There also needs to be an effective way of inserting the siRNA. The method which would be most effective for this is currently is to use a virus as a vector. Although there are many ethical issues involved it has already been highly researched and therefore could easily be applied to Bluetongue disease which would mean that the treatment would be

available sooner. There are also less likely to be problems with the treatment as it is highly researched. Liposomes could also be used to treat the disease but currently the best option is using a retrovirus.

## REFERENCES

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Sue Hocking, Pete Kennedy *et al.* (2008) OCR Biology, Heinemann

Figure 1 - image of a plasmid [www.abpisschools.org.uk](http://www.abpisschools.org.uk)

Figure 2- image of a liposome [www.bioteach.ubc.ca](http://www.bioteach.ubc.ca)