

Foot and mouth is a virus. Is siRNA a 'cure'?

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PASS WITH MERIT

**Research paper based on the
Pathology Lectures at
Vet-Medlink 2009**

ABSTRACT

Foot and Mouth is a highly contagious disease that mainly affects livestock and it has a significant bearing on the economy. The single stranded ribonucleic acid, (RNA) within the virus is synthesized by the host cell. The host cell then lyses and the new viruses are released. If a specific small-interfering RNA (siRNA) is introduced at the right time during the viruses' cycle can we cure this disease and hence eradicate Foot and Mouth? The economic benefit would be felt by many countries worldwide. Substantial livestock and to a much lesser degree human life would be saved. The potential to not only save livestock but to avert a human and economic crisis intensifies significantly if we consider the far reaching effects of virus mutation. A mutation could not only defy the current livestock vaccination programme but could possibly infiltrate large numbers of humans.

INTRODUCTION

The use of RNAi in Post-transcriptional gene silencing, (PTGS), was only recently discovered and currently could impact science further than anything has done before. It is a means to suppress genes and so could theoretically 'cure' many diseases of both viral and genetic origin.

PTGS was discovered, through an unexpected observation made whilst trying to deepen the purple colour found on the Petunia flower. A gene that had been found to produce pigment was artificially inserted into the Petunias and rather than a deeper purple being produced the Petunias became variegated⁽¹³⁾. The scientist responsible for the experiment, Rich Jorgensen, named this co-suppression as both the endogenous gene and the artificially introduced pigment causing gene had been suppressed. However, at this stage no one knew what caused this gene silencing effect; especially in the post-transcriptional gene silencing level⁽⁴⁾. This sparked a large number of experiments. Yet the answer was first demonstrated in *Neurospora*, 'Carlo Cogoni, Nicoletta Romano & Giuseppe Macino, (1994)'⁽⁶⁾, the gene silencing could be transferred between nuclei in heterokaryotic strains. Palauqui and colleagues confirmed that, this method of gene silencing was also present in plants. Work was then carried out in nematodes and flies by 'Fire and Mello, Nature. (1998)⁽¹⁾'. From here many scientists and research groups have meticulously carried out further work to investigate why double-stranded RNA results in gene silencing. Rapidly our understanding of the RNAi pathway increased tremendously, as a result of a few key discoveries. The first one was by Baulcombe and Hamilton where by RNAs of around 25 nucleotides under went co-suppression that were absent in non silenced plants. Further research, involving *Drosophila*, added to this picture. Yeast was used to produce dsRNA and these were then microinjected into *Drosophila* embryos⁽⁴⁾. Over the years this form of RNAi strategy has been used as a reverse tool. When dsRNA was added to *Drosophila* in vitro systems it was processed into siRNA and these were shown to cleave mRNA and so prevent Translation. All of these investigations have provided us with an understanding of RNAi and its uses with regard to gene silencing.

The RNAi pathway is present in all eukaryotes. Endogenous double stranded RNA; (dsRNA) invades the cell and in doing so activates the ribonuclease protein, Dicer. This results in the dsRNA being cleaved. This produces short dsRNA of 21-25 base pairs. Short dsRNA is also known as siRNA; this then activates the RNA induced Silencing Complex, (RISC). The RISC can then cleave messenger RNA, (mRNA) which is bound to the siRNA. One of the two strands degrades and the other binds to the Argonaut and directs the gene silencing⁽¹¹⁾. The activated RISC opens the strands and the specific fingerprint is copied. This would then result in the matching RNA being destroyed. This pathway is relevant to not only human medicine, by which cancer cells may be destroyed but also in veterinary medicine. The pathway can be used as a preventative therapy, or may even be developed into a cure for a number of diseases, including the Foot and Mouth Disease, *Aphthae epizooticae*⁽⁸⁾.

The Foot and Mouth virus is part of the Picornaviridae family. Although this virus is most commonly associated with livestock, it can also present itself in wild animals. These include ruminants (Deer and some zoo animals) Hedgehogs, Elephants and although to a lesser extent camelids including Camels, Alpacas and Llamas ⁽⁸⁾. The latter two, which although develop some mild symptoms, do not pass it on to others. Consequently, many species of animals all around the world are at risk to the possibility of contracting of the foot and mouth disease. This inevitably affects many countries and there is no distinction between developing countries and the developed countries, the Middle East is one area of the world affected. As a result, a number of political, economical issues are raised; principally that the Foot and Mouth Disease is a highly contagious and once it has appeared somewhere in a country an epidemic is not unforeseeable. The virus, once it has entered the body, comes into contact with a host cell and binds to its receptor site ⁽¹⁵⁾⁽⁵⁾. Currently there are Foot and Mouth vaccines. However, due to the large number of strains of the virus, these are not proving as efficient at controlling the disease, owing to the different base sequences within the various strains. They are a tentative preventative at the moment. This is because the Foot and Mouth Disease is caused by the virus, picornaviridae; it is constantly mutating and evolving. So base sequences can change within the viral RNA sequence and so the vaccines are ineffective. The current vaccines must be highly specific to overcome the huge variation of the seven known strains of the Foot and Mouth Disease ⁽⁸⁾.

This is where the RNAi pathway may become of some use. If cells along the RNAi pathway could recognise foreign RNA, such as that present in the Foot and Mouth virus, they could prevent the mRNA from the foot and mouth virus being replicated. This would be done via siRNA and the RISC. The mRNA would be cleaved in two and so no complete translation can occur. Hypothetically, this could be a much needed cure to the disease that has caused seven epidemics since 1914 resulting in millions of animals being slaughtered.

DISCUSSION

There are many possible developments of RNA interference, (RNAi) many of which are currently being investigated. However, I am interested in how we can effectively 'cure' the Foot and Mouth Disease, either temporarily or permanently.

One possible method, is by stopping the messenger RNA, (mRNA) of the virus synthesising and in doing so reduce the numbers of the Foot and Mouth virus and also the viral genomes; (the nucleic acid which contains all the genetic codes ⁽¹³⁾). In order for the mRNA to be cleaved the RNA-induced silencing complex (RISC), has to be activated. This is done by double-stranded RNA entering the cell, and activating the enzyme, known as Dicer. This enzyme then cleaves the endogenous double-stranded RNA into shorter dsRNA also known as small interfering RNA, (siRNA). These shorter dsRNAs of 21-25 base pairs long then activate the RISC. Each fragment produced by the dicer enzyme could form a functional small interfering RNA, (siRNA). However, only one of the two strands becomes a guide strand, also known as the antisense strand, which the Argonaute protein from the RISC, binds to. The other is degraded into its separate nucleotides ⁽¹¹⁾. The siRNA targets the mRNA and cleaves it in the middle. Consequently, this prevents Translation and so no new proteins can be made at the ribosomes. This is illustrated in Figure 1 ⁽²⁾ and an alternative example in Figure 2 ⁽³⁾.

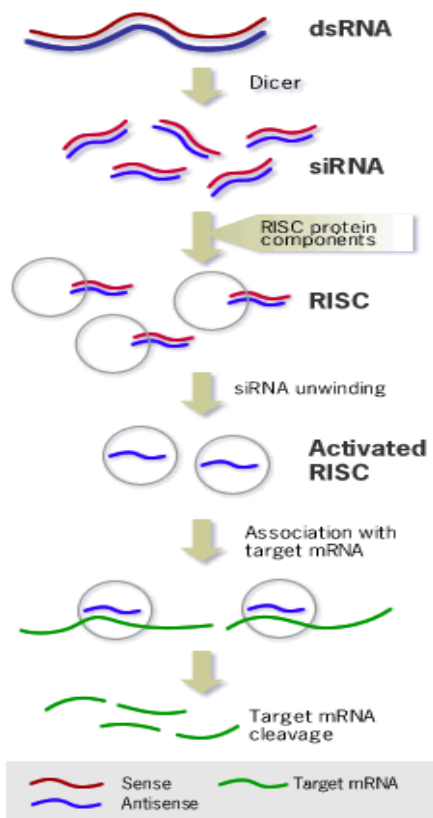
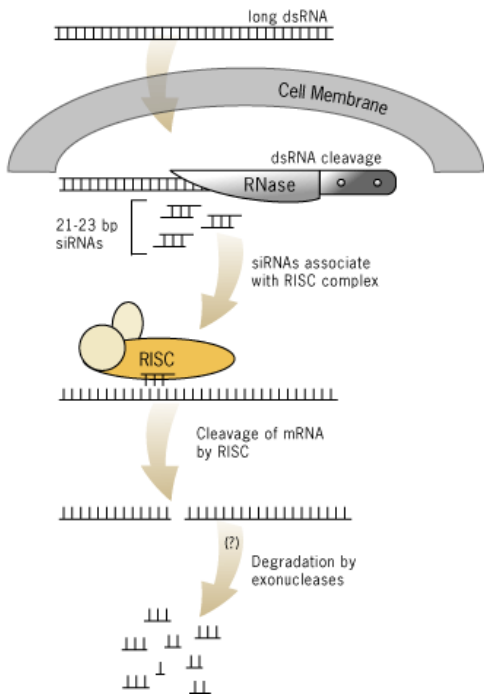


Figure 1
←

Figure 2
→



The RNAi is an efficient pathway due to RISC and its enzyme, which is able to catalyse a number of rounds of RNAi in vivo. In theory this could mean via the use of RNAi we could provide a permanent cure.

Within the Foot and Mouth virus the RNA is single stranded. Hence, in order to activate the RNAi pathway we need to introduce the short double strand RNA, siRNA, with a matching fingerprint to the RNA. This would then become incorporated into the RISC, where by one strand becomes a guide strand, known as antisense RNA, for gene silencing. The antisense RNA is complementary to the mRNA, ⁽⁷⁾⁽¹⁴⁾ within the virus which is needed for the viral replication. Once the complementary mRNA and siRNA have bound to each other then the mRNA is cleaved, consequently the virus is no longer able to replicate. In fact, all matching RNA is destroyed. This would mean that the viral mRNA is turned off in any other cell that has been invaded by the Foot and Mouth virus.

However, a major problem with Foot and Mouth is the incubation period. Like all viruses the Foot and Mouth virus mutates rapidly once it is inside the host cell. Hence the Foot and Mouth Disease, has an incubation period of 2-12 days ⁽⁸⁾. As a result it is difficult to know when the siRNA with the specific fingerprint for the Foot and Mouth virus needs to be introduced, in order to activate the whole process. This leads onto another question. If, for example, a farm is believed to have a Foot and Mouth outbreak upon it, should all the animals be administered with siRNA, in order to stop the viral replication? As once the siRNA has been incorporated into the RISC complex all matching RNA is destroyed. It would effectively remove all chances of the animals on the farm developing any more of the serious symptoms. However, not all the animals will have contracted the disease, what side effects might this action have upon them? Subsequently, in this method the siRNA would be used as a therapy. We could then extend this idea further. If the aim is to eradicate the Foot and Mouth Disease, should we use the method of introducing siRNA to activate the RISC complex as a preventative therapy? However cost and administration have to be taken

into account as outlined below. In other words, when all livestock reach a certain age, should they be administered with siRNA with a specific fingerprint to the Foot and Mouth viral RNA? This age of administration, would most likely have to be fairly young as the Foot and Mouth Disease affects young animals more severely than older ones. Not only is it dire that this disease affects livestock, but the fact that it is more severe in young animals is a lot more problematic. The youth of these animals means that economically they have more worth than older ones, as they are the ones to provide a country with the meat and dairy produce. Currently, if a farm is believed to have the Foot and Mouth Disease all the animals on it and in the surrounding area are culled; in order to stop this highly contagious disease spreading. This has resulted in devastating economic results and a great deal of unnecessary slaughter. The RNAi pathway could stop this.

Less slaughter of animals as a result of the Foot and Mouth Disease would mean that fewer animals need to be bred, in order to provide a country with the required products. This could mean that it would be possible to provide more food to developing countries. Also, during the 2001 Epidemic in the United Kingdom, the number of animals slaughtered resulted in the necessity of mass graves. This meant that land that could have been used for arable farming was in fact used for a graveyard. This results in less crop production for the population. There are also implications for the Health and Safety of the population, due to the incubation and bacteria and so consequently disease.

The Foot and Mouth Disease as previously mentioned is highly contagious, and can pass from species to species. This could mean that in a developing country where many economies are extremely fragile the disease could prove catastrophic. For example, a wild ruminant could contract the disease and pass it on to an animal that in a farmer's herd. This disease would then spread through out that herd; when these animals went to market, it would then spread through out all those animals present who would then return to other herds, and so on. The disease would infiltrate the country's farming community. Due to the siRNA being a recently discovered mechanism it may be extremely expensive so these farmers would mostly cull their animals. Yet, if the siRNA only needed to be introduced once to an animal to produce long lasting immunity then surely that would be a better option? The siRNA 'silences' a gene and so the disease cannot spread, and the farmer has not lost any animals. However, before this method is used we need to study how long the siRNA stays in the cell. If it does not stay long, then persistent administration of the siRNA needs to occur. Equally, if the siRNA stays a long time in the cell, then administration needs to be less frequent.

Another, issue concerning Foot and Mouth is Animal Welfare. Currently, another possible excuse for culling animals on a large scale is the animals' welfare. Foot and Mouth results in painful blisters mainly found in the mouth and on the feet. These have been known to restrict the animals' movement and restrict their eating and so contribute to weight loss ⁽⁸⁾. Not only are the swellings painful but they can burst and result in septicaemia, and secondary infection. Mature males, who contract the disease can experience swelling in their testes. This is why the foot and mouth disease can also be transmitted in cows by Bulls infected semen ⁽⁸⁾. Thus, using the RNAi pathway, we could eliminate this problem and the need for quarantine and destruction of many animals.

However, the use of RNAi to be used as a treatment for any type of disease has not been used yet. This means that it is a 'grey' area as to whether or not the Foot and Mouth virus could genetically mutate and so form a new fingerprint. A mutation, is highly likely as viruses are known to constantly mutate to become resistant to antibiotics, so why not for RNA in the virus? The viral mutation could then prove a

greater danger to humans, as currently human cases are rare. However, if we can stop the virus mutating through the use of siRNA, which cleaves the mRNA, we would stop Translation. If we cleave the mRNA in the animal strain of the Foot and Mouth Disease then the problem may never arise. Currently, humans cannot employ the RNAi defence as the introduction of the dsRNA to trigger the response, in fact triggers an interferon based inflammatory response. This is when the body's cells have their own defense mechanisms activated and so they hinder any foreign bodies trying to multiply and grow inside the human cells ⁽¹¹⁾. Therefore, if a serious mutation did occur in the Foot and Mouth Disease's genetic code, and it became a zoological disease, we would have a problem. This is because the foot and mouth virus has single stranded RNA ⁽⁸⁾ and in humans it is double-stranded RNA that triggers the interferon response. Therefore, the Foot and Mouth virus is, to an extent, suitable to infect humans successfully. We know how the dicer enzyme and RISC help to cleave the mRNA and both are present in humans. However, in order to trigger the RNAi response we need to insert dsRNA into our bodies in order for the RISC to copy the fingerprint and so allow all matching RNA to be destroyed including the Foot and Mouth Disease. However, the insertion of dsRNA brings about an interferon response in humans. In order to combat this problem it may be possible to adapt drugs like immunosuppressant used after organ transplants to halt the body's automatic response to the dsRNA. This would allow the RNAi pathway to be activated and the specific fingerprint of the siRNA recognised. The RISC would then destroy all matching RNAs including the mRNA, and so halt transcription. The reason I suggested the use of immunosuppressant is because these prevent T and B cell production. These are used in the bodies overall defence against attack and the interferon response in the cells, is their own defence against attack. The current reported symptoms of the Foot and Mouth Disease in humans are: malaise, fever, vomiting, red ulcerative lesions of oral tissue and vesicular lesions ⁽⁸⁾, but these could be greatly extrapolated if the virus mutated in such a way.

The whole idea of gene silencing is a social issue. It is a form of genetic engineering and many people oppose this. We are manipulating genes and controlling their outcomes. We are attempting to stop nature in order to further life. Some religious groups especially, think that as a race we are attempting to imitate God. They believe nature should be allowed to take its course. However, the counter argument to this is another ethical issue. Around the world millions are facing starvation. By stopping this disease we, may be able to provide more meat and/or land for crops and so we could send more produce to aid these people.

Every time Foot and Mouth appears on a large scale Trade Bans are introduced ⁽⁸⁾. These prevent the sale of meat from a country for a specified time. Yet, even when these restrictions are raised many people will still not buy the produce, "just in case". This can leave a country's economy struggling for many years after the disease.

CONCLUSIONS

I believe that through the use of the RNAi pathway, Foot and Mouth in animals could be eradicated. This is because; the majority of the ground work is already present and would enable this to be done. The RNAi pathway is catalysed by the RISC enzyme. Therefore, by introducing only a small volume of the siRNA we could potentially stop the disease progressing within the animal and prevent the spread in the local and the national populations. However, this will be easier to implement in developed countries as they are wealthier, so manufacture and distribution would be easier and

quicker. The problem of Foot and Mouth would remain present in developing countries as they are the converse of MEDCs. This would mean that the virus would still be able to mutate, possibly to an extent that would have a greater bearing on human life. This leads on to another problem. The virus triggers an immune response in the human body, when dsRNA is introduced. The dsRNA is needed to activate the RNAi pathway and so 'silence' the viral RNA and halt transcription. Therefore, testing needs to be thorough to ensure the virus does not mutate faster than the treatment can be developed. The use of immunosuppressant drugs could be a temporary answer for the interferon problem, until science has found another alternative route.

By 'curing' the Foot and Mouth Disease, an economic burden will be lifted. Also, there will be less need for trade bans upon produce from countries that have previously been infected by the disease. The 'silencing' effect on the gene will mean that animals are still able to enter the food chain. However, what needs to be investigated further is when the siRNA should be administered to the animals in order to be effective. If a compulsory administration was enforced, then we would need a method of distinguishing between the animals who needed the siRNA due to contracting the Foot and Mouth Disease and which ones did not. This could prove difficult due to the incubation period.

Ultimately, I believe that the use of RNAi as a 'cure' for the Foot and Mouth Disease is highly possible. However, before any programme can be initiated, details of the cost would need to be ascertained and the knowledge of which animals would receive the siRNA and when, researched. These issues require discussion in order for a decision to be made. Through testing, the dosage for each animal species needs to be discussed and whether the siRNA should be administered either as a tablet or an injection. Finally, has the technology and research reached a point where the manipulation of the RNAi pathway will produce an effective and worthwhile answer against the battle of Foot and Mouth Disease?

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