

IMPROVED SYSTEMS FOR THE IN VIVO DELIVERY OF
dsRNA

BY
DAVID MCBRIEN

PASS WITH MERIT

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT VET-MEDLINK 2009

Abstract

RNA interference (RNAi) is the specific gene silencing caused by double stranded RNA (dsRNA), RNAi is delivered to the body as small interfering RNA (siRNA) that are 21-23 nucleotides long. Since RNAi was discovered in 1998, it has been induced in nematodes, drosophila and vertebrates. Delivery problems, particularly in mammals, have arisen since its discovery. A novel delivery system using capsids derived from envelope virus grown in cultured cells from the individual to be treated is proposed, in this way any potential immune response would be reduced. Ethical issues in the area RNAi are also discussed.

Introduction

RNA interference (RNAi), discovered by Fire et al (1998), has been shown to be a useful tool for investigating gene function and drug development. Companies such as 'Sirna therapeutics' have been looking into the use of RNAi in problems like age-related macular degeneration, and they have had some success in this. The diagram below (figure 1) outlines how RNAi works, and how double stranded RNA (dsRNA) is utilised. RNA interference was induced in nematodes 'Fire et al (1998)' it has been induced in drosophila 'Kennerdell et al (2002)' and in vertebrates 'Wianny et al (2000)'. Due to the potential of RNAi, such as the efficiency and the relatively low cost it has become a leader in new methods to tackle disease. Since the discovery and development of RNAi areas have emerged in medicine where it could prove useful. Three prominent areas of medical research are dominant inherited disorders, cancer, and infectious diseases 'Kim (2003), Wall et al (2003)'. The specific nature of RNAi make it particularly useful as a genome screening tool 'Paddison et al(2004)'.

A major problem with RNAi technology is the delivery of the dsRNA because, unlike plants, mammals do not have dsRNA and when long chain dsRNA is introduced into the body it provokes a complex interferon based inflammatory response that shuts down protein production. 'Lacasse (2006)'. RNAi can be used in research to discover the function of the products of specific genes, by knocking them out. In medicine RNAi is used to knock out the expression of a particular disease causing gene. This paper will propose novel methods to deliver dsRNA to particular cell types without triggering an immune response it will also discuss some of the ethical issues that these new techniques could raise.

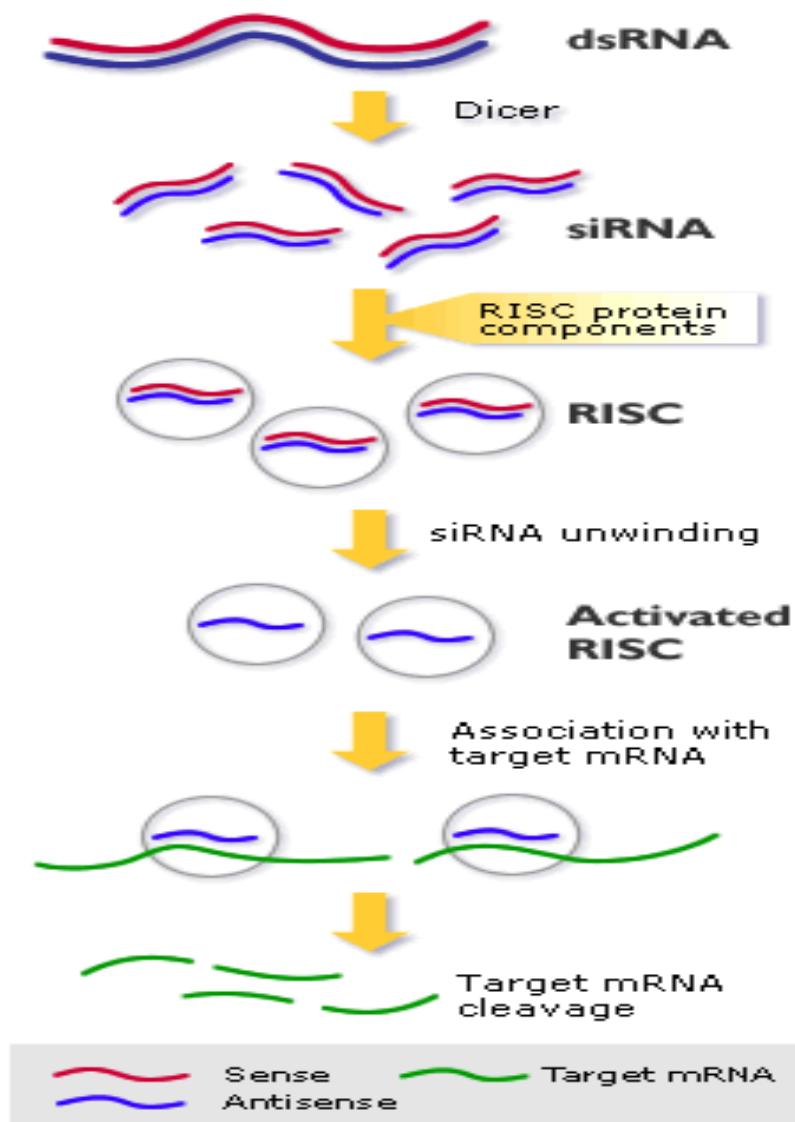


Figure 1.

Mechanism of RNAi abbreviations:
 dsRNA (double stranded RNA), siRNA
 (small interfering RNA), RISC (RNA
 induced silencing complex), mRNA
 (messenger RNA)

Discussion

Delivery

Local delivery has been executed in certain uses of RNAi such as in age-related macular degeneration where it is administered directly into the eye, and similarly in respiratory syncytial virus where the RNAi is administered via inhalation. These methods are fine for places where local administration is possible. There are many theories and developments of how the RNAi can be delivered such as using low molecular weight polyethylenimine (PEI) which effectively stabilises the RNAi for delivery 'Urban-Klein (2005)'. A major problem with RNAi delivery is that siRNA only has a half life of 1 hour in human plasma. RNA also cannot penetrate cellular membranes, which is a significant limiting factor on RNAi delivery. Retroviruses were used as an early vector in RNAi and have shown that there is potential in vitro 'Devroe et al (2002)' however there is a risk that as it integrated with the hosts genomic DNA it can be mutagenesis and carcinogenesis 'Hesketh (2007)'.

It is known that viruses are very efficient at delivering nucleic acids across cell membranes unfortunately they often elicit immune responses that harm the host.

The proposal is that use of viral capsids is made; these are the proteins shells that normally contain the viral genetic material. Molecular biological techniques could be used to remove all the genes but those that code for the capsid proteins and this will mean that there will be less pathogenic effects, as a fully competent virus is not being used. However, viral capsid proteins are still efficient at both taking up small sections of dsRNA and fusing with mammalian cells and delivering RNA to the cells cytoplasm. This would greatly reduce the chance of a pathogenic effect. There have been a few experiments using a similar technique for example such as 'Yin et al (2010)'. Unfortunately there would still be the risk of the host undergoing an immunogenic response to the virus protein. I propose the use of capsid proteins from envelope viruses (these viruses have an outer membrane derived from the host cell 'Lin et al (2000)'). It could be possible to remove some cells from the individual to be treated, white blood cells might be useful for this, and they would be cultured outside the body to increase their number. The cells could then be used to grow the viruses that have been genetically manipulated to just produce capsids, however if they were envelope virus they would be covered in the cell membrane, as they leave the cells. This would be derived from the patient's own cells and, therefore, avoid triggering a general immune response when put back in via an injection.

The second major problem in delivering dsRNA to a patient is targeting it to a particular cell type. All cells have a distinctive range of proteins on the cell membrane. It would be necessary to experiment with inducing the expression of receptor proteins on the capsid surface which would recognise a particular type of cell membrane protein. The genes for these proteins would be inserted into the viral genome that produces the capsid.

Ethical

Finding an effective and reliable delivery method for siRNA is vitally important for the future of RNA interference; however as with everything in science there are some ethical issues involved. The four principles of biomedical ethics, 'Beauchamp and Childress (2005)'. Principle one states that there should be respect for autonomy, this particular principle does not largely apply for RNA interference except that candidates for testing, must be aware what they are participating in. The second principle, Beneficence, ensures that the balance between risks and costs is looked at and is in the patients best interest. Non maleficence which states that any harm caused to the patient should not be disproportionate to the benefits of the treatment, is the third principal. An example of when this failed is when Alain Fischer treated two paediatric patients with gene therapy for severe immune deficiency syndrome(x-SCID) and they developed leukaemia following the use of retroviral delivery systems 'Hesketh(2007)'. Isolating the capsid protein from the virus avoids any viral associated problems within the body, which is how this delivery method would comply with the third principal. The fourth principal, justice is to keep things fair between all participants and patients. Justice in regard to RNA interference therapies is as important through the experimental phases as they will be through the drug phase, researchers may want to exclude people with a disease from the trials as they want to see the research result, however since the 1990's focus has been on therapeutic trials, this gives people fair access to clinical trials.

Beauchamp and Childress stress that no one principle is greater than another and the principles taken into account depend on the context of the situation. The principles do occasionally contradict other principles and it is up to the physician or researcher to make the choice, which then reintroduces the issue as to whether certain decisions are ethical. If a child was born with Celiac disease and the capsid delivery system made it possible to knock out the gene responsible would this be ethical, similarly if a puppy was tested positive for progressive retinal atrophy, there is no clear cut answer to these questions.

Importantly with any gene altering techniques, such as RNA interference there are religious implications to be taken into consideration. Certain people do not agree with altering what we were born with, as it is against 'Gods' wishes. Other ethical implications involve test subjects, whether it is ethical to test on animals, in the case of RNA interference there are not a lot of animal tests as a lot of this is being cultured in vitro, and when in vivo most companies are using cell cultures rather than live animal tests. With RNA interference still being experimental in a lot of areas of disease, it is hard to argue all the ethical implications of RNA interference as we do not know what, if any gene alterations can have in the future.

Conclusion

This paper has shown the features of protein capsid delivery systems and how these can be utilised for the delivery of siRNA. Problems that are now faced with using Capsids from viruses include how to get the dsRNA to recognise and be accepted into a particular cell, this could be achieved by using the tests done on membrane proteins to achieve a complementary fit on the capsid delivery system of RNA interference and how to deliver the siRNA. Further investigation will be need to be done on cells, to allow the viral capsid delivery to be effective.

As far as ethics are concerned there will be ethical issues with RNA interference, due to harm caused to patients during the experimental stages, and when RNA interference has become a useful tool for curing cancer and hereditary genetic disorders. The different delivery methods, are all being experimented with to deliver the siRNA, there are bound to be more accidents like Fischer's two paediatric patients who developed leukaemia after using a retroviral delivery system. As long as the four principles of biomedical ethics are followed, and common sense is used in regard to testing and use of RNA interference, in animals and humans then ethical problems will be greatly reduced in the delivery and use of RNA interference.

References

Beauchamp and Childress; Principles Biomedical Ethics, OUP, 5th edition (2001)

(<http://www.ethics-network.org.uk/ethical-issues/ethical-frameworks/the-four-principles-approach>)

Devroe, E and Silver, P.A, Retrovirus-delivered siRNA (2002)

(<http://www.biomedcentral.com/1472-6750/2/15/>)

Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391, 806-811.

(http://www.pauljanssenaward.com/media/Nature_Mello&Fire%20paper.pdf)

Hesketh, C. siRNA delivery methods (2007)

(<http://www.ionchannels.org/content.php?contentid=8>)

Kennerdell, J.R., Yamaguchi, S., Carthew, R.W. (2002). RNAi is activated during *Drosophila* oocyte maturation in a manner dependent on aubergine and spindle-E.

(<http://genesdev.cshlp.org/content/16/15/1884.abstract>)

Kim, V.N. (2003). RNA interference in functional genomics and medicine. *J. Korean Med. Sci.* 18, 309-318.

(<http://www.ncbi.nlm.nih.gov/pubmed/12808314>)

Lacasse, E. (2006) Apoptosis Control Based on Down-Regulating the Inhibitor-of-Apoptosis (IAP) Proteins: Xiap Antisense and Other Approaches.

(<http://www.springerlink.com/content/gu83127603l51u31/>)

Lin CL, Chung CS, Heine HG, Chang W. Vaccinia virus envelope H3L protein binds to cell surface heparan sulfate and is important for intracellular mature virion morphogenesis and virus infection in vitro and in vivo.

(<http://www.ncbi.nlm.nih.gov/pubmed/10708453>)

Urban-Klein B, Werth S, Abuharbeid S, Czubayko F, Aigner A. RNAi-mediated gene-targeting through systemic application of polyethylenimine (PEI)-complexed siRNA in vivo.

(<http://www.ncbi.nlm.nih.gov/pubmed/15616603>)

Wall, N.R. and Shi, Y. (2003). Small RNA: can RNA interference be exploited for therapy? *Lancet* 362, 1401-1403.

(<http://www.ncbi.nlm.nih.gov/pubmed/14585643>)

Wianny, F. and Zernicka-Goetz, M. (2000). Specific interference with gene function by double-stranded RNA in early mouse development. *Nat. Cell Biol.* 2, 70-75.

(http://www.nature.com/ncb/journal/v2/n2/full/ncb0200_70.html)

Yin Y, Cao LY, Wu WQ, Li H, Jiang Y, Zhang HF. Blocking effects of siRNA on VEGF expression in human colorectal cancer cells.

(<http://www.ncbi.nlm.nih.gov/pubmed/20205278?dopt=Abstract>)