

COULD NANOTECHNOLOGY DESIGNED FOR CANCER
DRUG DELIVERY BE USED TO HELP CURE HIV?

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PASS WITH MERIT

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Abstract

There is currently a lot of research being carried out into the use of nanotechnology to help deliver chemotherapy to tumours in cancer patients. This would be beneficial as it would dramatically reduce side effects of cancer treatment. This paper is an investigation into the use of this technology to help cure HIV which is currently an issue worldwide. It looks at further developments into treatment that could be sparked by the use of nanotechnology. Potential difficulties with this suggestion are also explored as well as ethical issues that arise from testing methods and the idea of deliberately placing toxic chemicals inside the body.

Introduction

Nanotechnology uses the idea of building small particles up from atoms. These structures have many applications in medicine, one of which is drug delivery. Nanoparticles can be used to transport drugs to the part of the body where they are needed without damaging other cells. In the case of cancer treatment, a particle can be used to transport cytotoxic chemicals to the tumour which would reduce damage to healthy cells and so greatly reduce the side effects of chemotherapy. It would also lower the fatality rate of cancer. There are several ways for nanoparticles to deliver drugs to where they are needed in the body; the drug can either be attached to the particle or enclosed within it.

One of the new developments is “Aurimmune” which is being developed by the researchers for Cytimmune, a drugs company founded by Lawrence Tamarkin, Ph.D. and Giulio F. Paciotti Ph.D. Their technology is based on the idea that particles can be made to measure 27Nm and would therefore only exit the bloodstream close to cancer tumours because the recently formed blood vessels supplying nutrients to them would have holes due to not being as developed as the rest of the circulatory system. As shown in Fig 1, the molecule consists of a newly developed type of fine gold particle, which (according to an article by Bidisha Mukherjee Bsc) is a therapeutic substance believed to reduce inflammation in the body. It is then attached to a Polyethylene glycol (PEG) particle. This component cannot be adsorbed onto cells and allows the particle not to be recognised by the body’s immune system. The cytotoxic drug used is Tumour Necrosis Factor (“TNF”). It works by binding to receptors in cells and activating caspase to change cellular proteins, causing apoptosis (death of the cell). TNF is used naturally by the body as the death of cells triggers the body to investigate and then start to destroy any invading pathogens.

Fig 1. The structure of the “Cytimmune” nanoparticle for drugs delivery

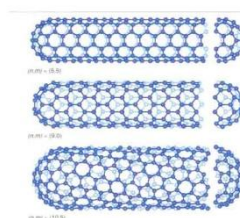
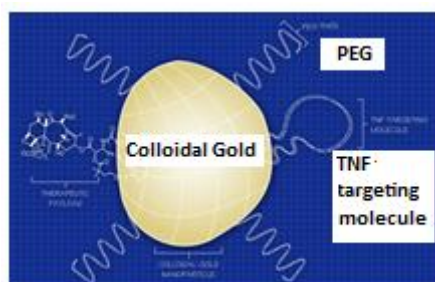


Fig 2- The structure of a nanotube with a cap

Another way to deliver drugs is to use carbon nanotubes. These were first discovered in the twentieth century by Paul Ehrlich who searched for a “Magic Bullet” to deliver drugs to the body with few side effects. Nanotubes are made when a 50amp current produces an arc between two graphite electrodes in helium and the vaporised graphite forms nanotubes. Graphite can also be converted into nanotubes by a laser or through the decomposition of a hydrocarbon gas near to a catalyst. The catalyst contains nanopieces of metal which are made bigger by the carbon with the metal at one end forming a cap; the end product of this is shown in Fig 2. Chemotherapeutic drugs are then loaded into the nanotubes and go into the patient’s body.

Some nanotubes are engineered to fit to folate receptors and have been used to deliver immune stimulants, lysosomes, and protein toxins. This process is illustrated in Fig 3. After binding to the receptors, the nanotube is taken into the cell by endocytosis and is then enclosed in a vesicle. Enzymes in the cell can release the drug either by breaking down the nanotube or removing the cap. One of the drugs used is cisplatin as it is a suitable size to fit inside the nanotube. It is a platinum drug which can enter cells by diffusion and helps inhibit DNA synthesis, meaning that the cell cannot replicate and the cell cycle stops. The drug also causes apoptosis of cells.

Fig 3 Drugs from nanotubes are taken in by cells

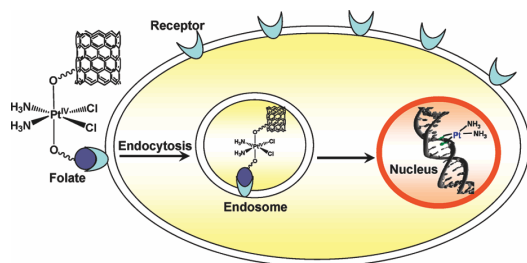
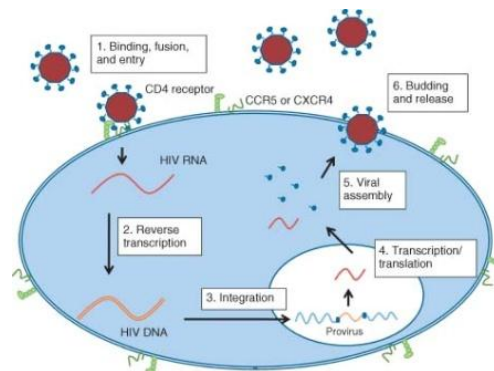


Fig 4- How the virus replicated in body cells



I think that this new technology could be used to aid research into finding a cure for Human Immunodeficiency Virus (“HIV”). This is a sexually transmitted virus that was passed on to humans in the last 50 years and is now a major worldwide problem, especially in developing countries. The viral envelope binds to T / CD4 cells and fuses with them, the envelope then disappears and the internal core of the virus is broken down, exposing the RNA. The RNA seroconverts into DNA and becomes part of the genetic information within the T cell meaning that whenever the T cell replicates, it produces more viruses. Fig 4 shows this cycle. The virus wipes out T cells, and therefore stops the immune response from being able to get rid of pathogens and so deaths are largely due to other infections. There are currently several accepted types of antiretroviral therapies (ART) that combat the illness by stopping the functioning of the virus/ infected cell. Entry inhibitors such as Enfuvirtide were first approved in 2003 and block the fusion of the viral envelope to the cells, so the virus cannot enter the T cells. The first types of ART drugs to be approved were reverse transcriptase inhibitors. They stop the conversion of RNA to DNA as RT catalyses this reaction. Protease

inhibitors bind to HIV-1 protease and stop it from splitting new proteins so they cannot form new viruses. Antisense oligonucleotides are strings of 2-20 nucleotides with a complementary shape to the viral RNA and can bind to it and inhibit replication. This can also be done using smaller fragments using a technique known as RNA interference where small sections of double stranded RNA can be cut using enzymes and then bound to the viral RNA. Targeting specific genes could stop the virus from being able to function or replicate. However, these inhibitors only target the virus when it has an active cell cycle and is replicating.

The major problem for doctors is that some of the HIV virus lies latent in a small proportion of T cells and can flare up after the rest of the infection has been treated. It is difficult to kill as there is no indication of which cells house HIV. Another problem is that potential medication to activate these viruses is toxic in great doses. My idea is that drug delivery nanoparticles could be used to transport activation chemicals to T cells, leaving the rest of the body unaffected.

Discussion

At the moment, researchers are investigating the use of histone deacetylase inhibitors to stimulate latent cells and cause them to actively replicate, meaning that they could be destroyed by existing antiretroviral drugs. DNA coils around proteins called histones. Amine groups on histones are positively charged and bind to the negative phosphate groups on the DNA molecule, causing it to condense. Acetylation neutralises the amine groups on the histone and therefore causes DNA to uncoil. Histone deacetylase removes acetyl groups from histones which would increase the positive charge on them and encourages bonding which again condenses the DNA. Inhibiting this enzyme will cause DNA expansion in viral cells, as show in Fig5. This causes DNA to be transcribed and stimulates the viral cell into its cycle and replication so that it can then be destroyed by ART drugs.

Fig 5- How histone deacetylase inhibitors affect DNA structure

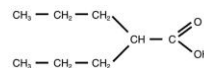
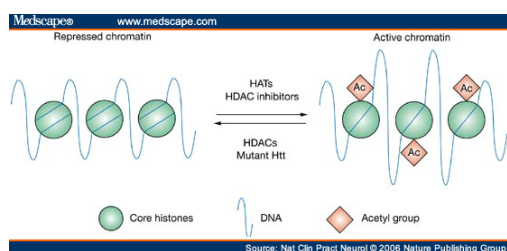
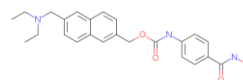


Fig 6- The structure of Valproic Acid

Fig 7- The structure of ITF2357



The first types of histone deacetylase inhibitor tested were found to be too weak or cause too many side effects as histone deacetylase is found naturally in the body and is important in the regulation of cellular growth and death. However, research is continuing and there are currently a few types of this inhibitor being tested. One of these is Valproic acid (fig 6); it has a double bonded oxygen atom as part of the molecule, like the acetyl group on the DNA histone. It has not yet been approved by the FDA and is still in phase II clinical trials. In 2005, Prof Margolis and his team tested the use of this chemical on four HIV patients who were receiving highly active antiretroviral drugs (“HAART”). They were also taking enfuritide to stop the spread of HIV within their bodies. The volunteers were given 500-750

mg doses of Valproic acid over three months. The results were very positive with a huge decrease-averaging 75%- of infected CD4 cells in three of the four patients and only slight reactions to the medication were experienced. This trial was then followed up by Margolis in 2008 when he observed the effects of taking 100mg Valproic acid on patients on normal doses of ART. Of the eleven volunteers taking the drug, only four showed a decrease in resting CD4 cells. The French ANRS EP 39 test observed the effects of Valproic acid on HIV patients over two years and found that the reduction of resting CD4 cells was insufficient to cure the HIV. More recently, researchers have started to investigate the use of ITF2357 (Fig7) and the results were published in the journal of immune deficiency syndromes in May 2010. P24 is a core protein of the HIV virus and so the efficiency of ITF2357 was tested against Valproic acid by the comparison of the amount of p24 in blood samples as this would indicate activated viruses. The concentration of p24 was much higher in the cells treated with ITF2357 than in the acid. I think that this could be due to the fact that there are more double bonded oxygen atoms on the branches of this molecule and so suggest that research is carried out to see if this can be increased. Using a higher concentration of the deacetylase inhibitor could also increase efficiency as there would be more molecules for the deacetylase to bond to. There is also the option of new types of HDACi and the development of others currently in trial. The possible successes of using nanotechnology could spark more research into these as it gives a new way for them to work in and higher doses could be used.

It may be possible to make a nanoparticle that binds to CD4 receptors of T cells- the same receptors as the virus- that could deliver Histone deacetylase to all T cells. This could awaken any dormant HIV virus, allowing it to be completely destroyed with existing drugs taken at the same time, such as enfuritide that was used in the experiments carried out by Margolis to clear any active HIV before testing. I think that it would be safer to use nanotubes in these experiments (rather than the gold particles) as white blood cells occur in the bloodstream so there is not such a specific point in the body for the particles to travel to. It would also be safer for the HDACi to be released only inside the cells as it has to come into contact with the HDAC to be effective whereas TNF works by binding to cell receptors and joining with other chemicals. I propose that scientists could work to develop a nanotube containing ITF2357 as it has so far had more positive results. Once inside the T cells, the cap could be removed by the cell and the HDACi would be released, causing any latent virus to activate. I imagine that this research could trial HDAC inhibitors at higher concentrations than in the earlier investigations which showed safe doses to be too weak or cause too many side effects as these inhibitors are also toxic to normally functioning body cells which also use HDAC. This is because nanoparticles could be designed to only deliver this drug to CD4 cells and therefore experiments of inhibitors could be carried out with higher concentrations. One possible issue with doing this is that the HDAC inhibitors could have a harmful effect on uninfected T cells. However, I feel that without the drug these cells are at risk of becoming infected and so being destroyed anyway. White blood cell transfusions would not be effective as the donated cells would not last long in the blood stream. Instead, Filgrastim, a drug that is currently used to help people with AIDs, could be used to stimulate the production of neutrophils from the bone marrow which would help to rebuild an immune system.

Some HDACi could leak out or diffuse into the bloodstream. Although this could be useful in clearing any surrounding infected white blood cells, it could also damage other types of cell. Scientists could make this less dangerous by making sure that only the right amount of HDACi was inserted into each nanotube to awaken the virus to limit amount that escapes into the bloodstream. Alternatively, they could do this by injecting some HDAC into the blood after treatment to clear any remaining inhibitors; the quantity would have to be calculated by further testing as adding too much could also damage cells. I believe that the overall effect of using nanotechnology would still be positive as initial doses are given straight to T cells. This application of nanotechnology would allow researchers to explore how effective HDAC inhibitors could be further and hopefully be able to cure HIV permanently in individuals in the future.

Very recently researchers in the Northwestern University in Chicago have managed to make neurons from stem cells that may be inserted into the brain. This success could help in the cure of HIV as it may also be possible to create the patient's own healthy white blood cells from stem cells to replace any that have been destroyed because of the virus or potentially due to the HDACi.

Some ethical issues are raised by the testing of nanotechnology and the idea of introducing toxic chemicals into the body. The first is that most of the testing of both nanocarriers and HDAC inhibitors is carried out in mice and tumours are artificially induced. This can cause pain and death to these animals and some people feel that this is cruel and against animal rights. In doing this, researchers have to consider morally if humans have the right to induce tumours on mice and weigh this against the possibility of saving people's lives with new findings. It also raises the question of who should decide what the right thing to do is. Another practical issue is that results obtained from animal testing are not always concordant with what would happen in humans. An example of this is TGN1412 which was developed in 2006 to cure leukaemia, rheumatoid arthritis and multiple sclerosis and had few noted side effects on animals. However it caused multiple organ failure in six volunteers at human trialling. This may be less of a risk for HDAC inhibitor testing as tests can be carried out on blood samples of infected patients. However, the new nanoparticles would still need to be tested for any effects on the body.

Another issue is that both TNF and HDAC inhibitors are toxic to the body and researchers have to take into consideration that this may have long term adverse effects on the body that are not yet known. Some people believe that it is immoral to put these chemicals into the body. Again, this has to be weighed up against the possibility of potentially curing HIV and AIDs.

A lot of problems with HIV occur in developing countries which may not be able to afford any newly developed medication as it can be very expensive and so funding may be reliant on charity. However this may benefit charities in the long run as it could reduce the amount of healthcare and financial needs of families after deaths due to AIDS. I also feel that it may be something that the rest of the world may be willing to help fund and the fact that it would be needed on such a large scale may also reduce costs.

Conclusion

This investigation has explored the possibility of using nanoparticles to help cure HIV. I have put forward the idea that they could deliver histone deacetylase inhibitors only to white blood cells in order to awaken any latent virus and allow it to be destroyed by existing antiretroviral drugs. This application is based on existing drug carriers which also aim to reduce the side effects of toxic medication on the body. The use of nanotechnology could be beneficial as it would allow research into the use of higher doses of these inhibitors and help to reduce potential toxic effects of this chemical in vivo and mean that more could be done to make sure that the virus is awakened without such a high risk of toxic effect on the body. However, further research would also need to be carried out into the possible effect of HDACi on healthy white blood cells and how this could be reduced. One way to do this could be to develop the use of stem cells to create new white blood cells for the patient. Although lots of research would need to be carried out, results could lead to a successful cure for HIV/AIDs which at the moment is a huge global issue.

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