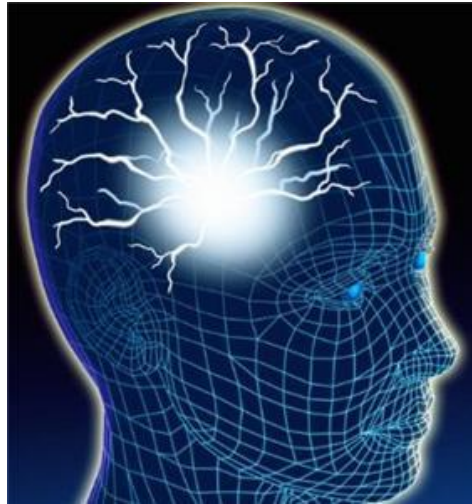


**NANOTECHNOLOGY BREAKTHROUGH: PENETRATION OF THE
BLOOD BRAIN BARRIER FOR THE TREATMENT OF PARKINSON'S
DISEASE**



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PASS WITH MERIT

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ABSTRACT

Nanotechnology has the potential to greatly improve public health through enhanced diagnosis and treatment of increasing numbers of conditions. At present, nanotechnology for drug delivery is extremely encouraging, especially in the direction of drugs across the blood-brain barrier (BBB). In this paper, we will explore the prospects of nanoparticle drug delivery across the BBB, beginning with a description of a current application: the use of nanoparticles to deliver antiretroviral drugs to the brain for the treatment of HIV. In our discussion, we will demonstrate how this research could be applied to transport dopamine across the BBB to the substantia nigra to treat the motor symptoms presented by Parkinson's disease (PD).

INTRODUCTION

The idea of nanotechnology was proposed by the Nobel Prize winning physicist Richard Feynman at the 1959 American Physical Society meeting at Caltech, where his "There's plenty of room at the bottom" speech described the potential manufacturing of devices using materials on an atomic and molecular scale. Today, nanotechnology is best defined as the branch of technology which involves the use of particles smaller than 100 nanometres, with a nanometre being equal to 1×10^{-9} m. The minute molecular size of these particles enables an extremely diverse range of uses, many of which form the basis of a wide complexity of medical treatments. This ranges from the use of aluminosilicate nanoparticles to reduce bleeding in trauma patients by absorbing water molecules in the blood plasma to drug delivery in cancer treatment involving the attachment of cytotoxic drugs to monoclonal antibodies.

Nanocarrier drug delivery systems work on the principle that the extremely small size of the nanoparticles allows them to bypass physiologic barriers such as the immunogenic response by being engineered to act as vectors for drug delivery to targeted tissues in the body. In particular, nanotechnology can be used to cross the blood-brain barrier (BBB) which poses hindrance to the absorption of drugs since its function is to regulate the movement of fluids from the blood to the brain parenchyma and vice versa, therefore providing protection of the brain and spinal cord from neurotoxic metabolites that may accelerate natural debilitation of the neurons.

Normal sized drug molecules have extreme difficulty in crossing the BBB since efflux pumps such as multispecific organic anion transporters and large numbers of high metabolising enzymes prevent drugs from penetrating the central nervous system (CNS). The brain tissue capillaries forming the blood-brain barrier lack fenestrations in their endothelium due to the cell surface membranes of adjacent endothelial cells being fused tightly together with several transmembrane proteins, such as occludins and claudines, meaning that there are very few gaps through which molecules may pass through. This forms the basis of the selectively-permeable nature of the BBB.

Antiretroviral drugs (AVRs) are unable to pass through the BBB. With an estimated 48 000 000 people currently infected with HIV, it is necessary to provide affordable, available and effective anti-retroviral therapy to as many people as possible. Generic drugs can cost as little as US \$88 per person per year but this cost is too great for many countries. Development of nanotechnology for drug delivery would potentially increase future AVR availability. HIV, being only 0.1 microns in diameter (180 billionth of a metre) can cross this blood-brain barrier as the CD4⁺ T lymphocytes and mononuclear phagocytes such as macrophages may conceal the virus within them and cross over the BBB. The virus then infects microglial cells of the brain by using their CD4, CD8 and chemokine receptors to bind and fuse to the cell surface membranes to allow entry into the cell. HIV-1-associated neurocognitive disorders (HAND) such as dementia may then occur due to there being extensive neuronal damage, particularly in the frontal cortex.

Antiretroviral drug molecules bind with proteins in the blood plasma. The larger molecular size of the antiretroviral-protein complex further restricts penetration through the capillary endothelium. The use of nanoparticles may overcome this predicament in a variety of ways. One way is to use a multiple

emulsion-solvent evaporation procedure to produce polylactic-co-glycolic acid (PLGA) nanoparticles (*Barichello, Morishita et al, 1999*) containing the antiretroviral drugs ritonavir, efavirenz and lopinavir. The research into the use of PLGA nanoparticles to transport the antiretroviral drugs into the CNS was conducted after the successful use of PLGA nanoparticles to target and consequently destroy cancer cells. In particular, research conducted by *Kocbeck et al (2007)* proved extremely promising since PLGA nanoparticles filled with cytotoxic drugs such as paclitaxel have been successful in the destruction of a significant proportion of cancer cells in trials. Each nanoparticle was coated with a monoclonal antibody (mAb) which acted as a homing ligand to ensure that only cancer cells were targeted by this drug. A ligand is a signal triggering molecule which has a high affinity with a site on a target protein. This specificity of the treatment has improved drug efficacy since an excess of the drug is no longer required for a sufficient amount of the drug to penetrate the infected area.

The PLGA nanoparticles used in antiretroviral drug delivery are biodegradable and protect the drugs that they contain from lysis within the body. They may also be engineered to optimise the sustained release of the drugs to reduce the amount required to fill the nanoparticles and the frequency of injection through a variety of molecular adaptations. The antiretroviral drugs can infiltrate the CNS by interacting with the endogenous transporters present in the endothelium and therefore move by endocytosis across the blood-brain barrier. Once within the brain extracellular fluid (BECF), the monoclonal antibodies on the surface of the nanoparticles will allow the specific areas of the CNS infected with HIV to be targeted, since these cells will present 'foreign' antigens on their cell surface membranes which will be complementary to the receptor site of the monoclonal antibodies (*Kohler, Milstein, Jerne 1975*). Therefore the antiretroviral drugs can be released to suppress the HIV replication to prevent further destruction to somatic cells that could potentially lead to disability in some form.

This application of nanoparticles and other similar research such as *Nanoart, neuroAIDS and CNS drug delivery by Nowacek, Gendelman et al (2009)* and *Impact of nanotechnology on drug delivery by Farokhzad, Langer et al (2009)* have proved that nanoparticles are capable of transporting and releasing drugs within the body, especially across the blood-brain barrier, for a variety of diseases. This is especially encouraging for the future use of nanoparticles for drug delivery for diseases that were previously impossible, such as neurological diseases like Parkinson's disease and Alzheimer's whereby the blood-brain barrier has presented a great obstruction to drug delivery into the CNS. That is, until now.

DISCUSSION

Parkinson's disease is a progressive neurological condition which affects 1 in 500 people, accounting for 120,000 people in the UK. Parkinson's is associated with age and with demographic ageing taking place across the economically developed world, the frequency of Parkinson's is expected to increase significantly in the coming decades. Motor symptoms of Parkinson's include bradykinesia (slowness of movement), rigidity and tremor whilst Parkinson's also impacts sufferers' lives by causing non-motor symptoms: tiredness, pain, depression and constipation. Parkinson's does not directly cause death but symptoms gradually worsen over time. There is no cure and the origin of the disease is not fully understood. However, it is known that synaptic function of neurons is disturbed and that a progressive loss of mid-brain dopaminergic neurons of the substantia nigra over several years results in development of a dopamine deficit. Dopamine is a key neurotransmitter in the brain.

At present, the main treatments for Parkinson's disease are drugs aimed at reducing the dopaminergic deficit such as L-dopa, monoamine oxidase B inhibitors and dopamine agonists. People with Parkinson's disease cannot simply take dopamine tablets because dopamine is too polar a molecule to cross the BBB. However, L-dopa (or levodopa to give it its generic name), the precursor of dopamine, can gain access to the brain via the large amino acid (LAT-1) transporter to which it is complementary in shape. LAT-1 transporters move amino acids such as phenylalanine and tyrosine across the blood brain barrier. Once

inside cells, L-dopa is absorbed by the surviving dopamine producing cells in the brain and is converted to dopamine, therefore improving nerve conduction.

Despite its usefulness in the treatment of Parkinson's disease, L-dopa is by no means a perfect drug. It must be administered in large concentrations to ensure that a small fraction of the drug will bypass the BBB. The large doses trigger unwanted side effects in other parts of the body. Writhing, jerking or free flowing movements and nodding (dyskinesia) can occur and there is a "wearing off effect" due to the slow rate at which the drug is absorbed. For the patient, it appears that the drug is exacerbating Parkinson's symptoms for a period of time after each dose. L-dopa is not 100% efficient because some of the levodopa in a given dose is converted to dopamine outside the brain where it is of no use and is potentially harmful. To reduce side effects and premature conversion to dopamine, levodopa is administered with several other drugs such as catechol-O-methyl transferase (COMT) inhibitors, which deactivate enzymes that change L-dopa to dopamine outside the brain, or it is combined with carbidopa or benzerazide to prevent nausea and vomiting. In turn, these additional drugs produce side effects.

This series of side effects could be eliminated by using a comparably more efficient nanocarrier drug containing the essential dopamine. Dopamine would make up one part of a nanoengineered complex whilst the surface could be customised on a molecular level to carry out specific functions to maximise efficacy. The large surface area to volume ratio of nanoparticles allows this customisation for multifunctionality. Functions include preventing side effects by shielding the drug, allowing the nanocarrier to cross the BBB, preventing clearance by the efflux transporters and targeting of specific cells after the nanocarriers has entered the brain extracellular fluid (BECF). Other features to consider when designing this nanocarrier will be whether it will have an immunological effect by activating neutrophils, causing opsonisation or causing inflammation. It must also be non-thrombogenic, and biodegradable.

Nanocarriers are polymers, amphiphilic lipids or solid colloidal particles which enclose targeted drugs. There are several different techniques by which drugs can be enclosed in nanoparticles and therefore there are numerous different styles of nanocarrier. Gold nanoparticles, ceramic nanoparticles, liposomes, solid lipid nanoparticles, polymer-drug conjugates and carbon nanotubes are just a few examples. It would be important to choose the design which would maximise the effectiveness of dopamine delivery across the BBB.

Dopamine could either be enclosed inside the nanoparticles sphere or linked to the surface, depending on the method of nanocarrier construction. The primary manufacturing methods are emulsion polymerisation, interfacial polymerisation, desolvation evaporation and solvent deposition. The preferential method in dopamine delivery to the CNS would be interfacial polymerisation as it encourages the formation of a nanocapsule shell. Once the drug is encapsulated within the shell, it is protected until it reaches the substantia nigra in the brain and degradation occurs. Protection is needed to prevent substances in the blood causing premature dopamine breakdown and more importantly, to avoid unnecessarily high concentrations of dopamine in the bloodstream. The nanoparticles produced in desolvation evaporation have a low capacity for drug absorption and those formed by emulsion polymerisation require large volumes of organic solvents so have the potential for toxicity (*Lockman et al 2002*). The ideal substance for the dopamine nanocarriers would be lipid as lipids are components of living organisms so the particles would be biocompatible and biodegradable. Size is also a vitally important characteristic as the lipid nanocapsules would need to be smaller than 100nm in diameter in order to cross the BBB.

Drug delivery across the BBB depends on making use of different pathways. The smallness of NPs allows them to cross the BBB by passive transcellular diffusion, if they are made lipid soluble, or by active targeting via receptor mediated endocytosis or absorptive transcytosis. For our purpose, receptor mediated endocytosis would be the favoured method because simply enhancing lipid solubility during chemical modification will result in an increased distribution of dopamine in all organs. Nanocarriers

which are conjugated to recognition and transcytosis-enhancing ligands that are capable of recognising brain capillary endothelial cells will allow targeted drug delivery.

In one path, the ligands bind to the receptors and the nanocarrier is gathered in specialised areas of the cell surface membrane known as coated pits which concentrate endocytic receptors and are coated with a lattice of the protein, clathrin. The coated pits invaginate to form coated vesicles. The receptor then detaches from the ligand conjugated nanocarrier, the nanocarrier degrades and the drug is released. The transferrin pathway is one particular endocytosis mechanism that could be exploited for nanocarrier transport. Iron obtained from the diet is carried around in the blood by the protein transferrin and enters cells via endocytosis mediated by the transferrin receptor. The brain takes iron from transferrin after entering cells via the transferrin receptor in the BBB endothelium. Friden (1994), Bickel et al (2001) and Moss and Morgan (2001) showed that antibodies which bind to transferrin receptors selectively target BBB endothelial cells due to the high levels of transferrin receptors expressed by these cells. More recently, transferrin has been recognised in the effective targeting of PEGylated nanoparticles containing azidothymidine (AZT) to brain tissue (*Mischra, V et al*). This shows that transferrin is a feasible targeting agent for the direction of dopamine containing nanocapsules across the BBB. Furthermore, this process will allow the nanoparticles to partially avoid the efflux transporters which remove particles and molecules from the BECF.

An interesting method of drug delivery which could be employed involves disguising nanocarriers as lipoproteins which can be absorbed by the brain capillary endothelial cells via receptor mediated endocytosis. Nanoparticles coated in the surfactant polysorbate 80, will absorb apolipoproteins B and E onto its surface so that it imitates lipoprotein particles (*Kreuter et al.*) This is called a “Trojan Horse mechanism” because the nanocarrier is concealed in a lipoprotein. Apolipoproteins are the protein element of lipoproteins.

Nanocarriers have many advantages over non-nanotechnology methods of circumventing the BBB. The original drug compound can be made lipid soluble and so can more readily cross the BBB using pro-drugs. However, neurotrophic factors and nerve growth factors cannot be modified in this way and still be capable of producing the intended therapeutic effect. In addition, increasing the lipid solubility of the drug may have a detrimental effect on drug clearance which is the fraction of theoretical volume no longer containing any of the drug concerned per unit time. Increased lipid solubility alters the drug half life (i.e. the time necessary to halve the plasma concentration of the drug.) If a dopamine containing drug was designed to use specific transport proteins in the endothelium for delivery without nanotechnology, the drug would have to have a specific molecular shape to ensure high affinity with the transport proteins. This restricts the potential structure of the drug so that it may not be as therapeutically successful. The process of molecular design of the nanocarriers with attached ligands allows specificity without modifying the drug or restricting its molecular shape.

Low dose solvents such as sodium dodecyl sulphate are used to open the tight junctions of the barrier. However, this allows plasma proteins and other unwanted molecules to leak into the BECF. Another potential method to sustain effective concentrations of a drug in the brain is inhibition of efflux transporters. Both opening the BBB and disturbing its equilibrating mechanisms would lead to CNS toxicity. Using nanotechnology, there is no need to manipulate the BBB's permeability and so introduce toxic substances to the brain's precariously delicate environment.

Transplantation or restorative surgery where dopamine-producing cells are implanted into the striatum is possible for Parkinson's patients, just as transplanting encapsulated cells secreting nerve factor into forebrain neurons for Alzheimer's patients is possible. However, this involves invasive neurosurgery, meaning that very ill or elderly patients who would not survive surgery could not be treated. The drug delivery route with nano-drug delivery is non-invasive. An issue with direct injection is that the drug will diffuse away from the injection site, limiting the effectiveness of the therapy. Moreover, this only applies

if the affected area is accessible, which is doubtful. Nanocarrier drugs have a slow and sustained drug release profile as they have long circulating properties and drug release from nanocapsules occurs predominantly by degradation or diffusion. Nano-devices which operate drug release over a specified amount of time can be installed into the CNS. Sustained drug release is crucial in treating brain tumours and would be useful in dopamine release in treatment of Parkinson's. This would allow the dose to be controlled as the amount of dopamine produced naturally in the brain diminished as the disease progressed.

Ultimately, using nanocarriers would make treatment of Parkinson's more economically efficient as fewer drugs would be necessary. Pharmaceutical companies could reconsider drugs that had previously been disregarded in clinical trials due to toxicity to the rest of the body, if they were retested when encapsulated in a suitable nanocarrier. Therefore, there could be a wider range of viable therapeutic options.

CONCLUSION

This discussion paper demonstrates the prospects of using colloidal nanoparticles to transport dopamine directly across the BBB for the treatment of Parkinson's disease. This has the capability to decelerate the decline in the synaptic function of the neurons, thus providing a more proficient means of treatment. Treatment would therefore be more economically efficient due to the sustained drug release from nanocapsules and the intricate molecular design in the form of ligands and other surface molecules which will allow for specificity of the treatment without altering the drug.

However, since the applications of using nanoparticles for delivery of other drugs are in their early stages, it is questionable whether our proposals would actually work. Existing research presents the possibility that dopamine could be directly administered to the patient; something that has seemed almost impossible up to now. On the other hand, the toxicity and safety of the nanocarrier-dopamine complex is uncertain and this issue will require further research and clinical trials over subsequent years.

The reticuloendothelial system (RES) would also act as a major obstruction to the use of the dopamine-loaded nanocarriers since the phagocytic cells would remove a large proportion of the nanoparticles from the blood before they had reached the BBB. The RES is a generalised phagocytic system located in all tissues. It is responsible for nanoparticle surface opsonisation, phagocytosis and ultimately clearance or retention in reticuloendothelial organs such as the spleen and liver which takes up more than 70% of injected nanoparticles. Although this problem could be rectified by reducing the diameter of the nanoparticles to around 4-8nm, this would consequently affect the volume of dopamine able to be transported in each nanocarrier. Immunosuppressant drugs could be supplied to inhibit the activity of phagocytes, allowing the nanocarriers time to move across the BBB by receptor mediated endocytosis, but this may cause the patient to become more susceptible to other diseases. The nanocarrier structure would have to be modified with surface absorbed polysaccharides and amphiphilic polymer chains such as PEGs to inhibit the electrostatic and hydrophobic interactions between the nanoparticles and the opsonisation proteins. However these modifications could have a detrimental effect on their effectiveness once inside the body.

In summary, using nanocarriers in dopamine delivery to overcome the quandary of limited access to the brain necessitates a lower dose of the drug required to produce an effective concentration of drug in the brain. This is due to the minute size of the nanoparticles allowing for penetration through the BBB for efficient drug accumulation at the targeted sites. Using nanotechnology in this way would improve the standard of living of Parkinson's patients by reducing the number of doses required and precluding the unpleasant side effects associated with L-dopa and its partner drugs which may make people unwilling to take their medication. The future looks extremely promising...

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