

Nanotechnology in Medicine

Applications in Drug Delivery to the Brain and Brain Tumour Therapy

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PASS

ABSTRACT

Nanotechnology involves materials and devices that are organised in at least one dimension on the nanometre scale, ranging from a few to about 100 nanometres, and nanoengineered materials in medicine are designed to interact with cells and their tissues at the molecular level. Developing methods for delivering drugs and other molecules to the brain and central nervous system, and overcoming some of the natural structures such as the blood brain barrier to give access for such substances is an important possible application of nanomedicine, where nanoparticles have a unique advantage over other existing techniques.

INTRODUCTION

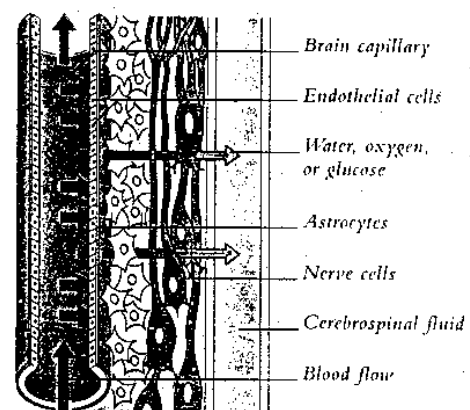
Nanotechnology is the study and manipulation of matter on an atomic and molecular scale, and involves the development of structures and devices sized within 1 to 100 nanometres in at least one dimension, a nanometre being one billionth of a metre. So nanomedicine is simply the application of nanotechnology in medicine, where highly clinically useful developments can be made to aid future diagnostics and therapeutic treatment.

Much potential lies in drug delivery: Currently many treatments to the brain involve very invasive methods such as drilling into the skull, and there is a scarcity of non-invasive techniques in delivering drugs to the brain specifically. Dye can travel from the spine to the brain, but by injecting drugs to the brain in this way by injecting into the spine and surrounding cerebral fluids is not only dangerous, but very painful for patients. Nanoparticles can offer a solution to this problem. Nanoparticles have properties that can improve drug delivery, for example their size allows cells to take up such particles whereas larger molecules – those which may have been drugs, may be instead cleared from the body. Often bodily enzymes may act against the transfer of many drug molecules too. Improvements in drug delivery would involve bioavailability, absorption, distribution, safety, efficiency factors. Drugs which may be cleared from the body quickly may force patients to take higher doses. Bioavailability involves the presence of drug molecules in parts of the body where they are most needed and will do the most good. Nanotechnology can improve these factors in improved drug targeting.

DISCUSSION:

THE BLOOD BRAIN BARRIER (BBB)

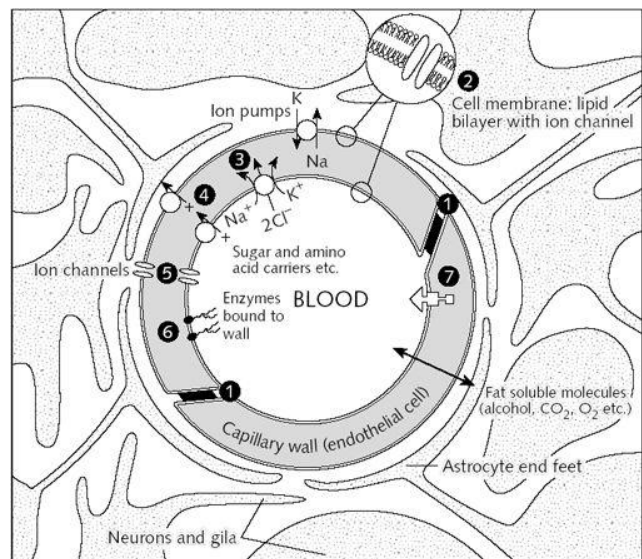
The difficulty in treating brain disorders arises from the idea of crossing and overcoming the blood-brain barrier. The blood brain barrier separates the circulating blood from the brain extracellular fluid, and consists of a semipermeable monolayer of endothelial cells that line the outside of all the capillaries of the brain. This obstruction does not occur in the rest of the body in the blood vessels and reduces the



permeability of these blood vessels in the brain. Although substances injected into the main bloodstream may be circulated throughout the whole body the structure of the blood brain barrier somewhat compartmentalises the brain from the rest of the body and so does not necessarily mean these substances will reach the brain tissues.

In blood vessels throughout the rest of the body, there are small spaces between adjacent endothelial cells which offer some degree of permeability, where substances can pass readily between the inside and out. In the blood-brain barrier, endothelial cells are fitted and sealed tightly together in tight junctions, acting as a physical barrier so substances cannot easily pass in this way. Capillaries are also surrounded by astrocyte cells, which offer biochemical support to these endothelial cells and also act as a partial barrier, and are also lined with enzymes that hydrolyse any unwanted molecules flowing through the brain, providing a more active defence. Because of this, the passage of molecules that are too large, ionic, hydrophilic solutes, and most pathogens into the cerebrospinal fluids that surround the brain are restricted.

The advantage of the blood brain barrier is that it provides protection, for example from foreign substances that may cause damage, from hormones and neurotransmitters in the rest of the body, and proves effective in protecting the brain from bacterial infection. It is also important in helping the brain maintain a constant internal environment, which is biochemically, unique to the rest of the body; For example in isolating it from disturbances in composition of fluids in the rest of the body in homeostasis due to infection or trauma. However this also poses the problem when beneficial diagnostic and therapeutic molecules are unable to gain entry, which would help combat neuronal disorders.



We can see that the presence of the barrier restricts a lot of movement of substance, but essential nutrients and compounds must pass to the brain for nourishment such as glucose and amino acids in respiration and protein synthesis, so the special carriers such as transporter proteins have developed within and out of the capillaries to transport these substances from the blood to the brain.

Mechanisms for drug targeting the brain involve either going through or behind the blood brain barrier. So for example, we can manipulate the special carriers within the vessels with the help of the brain to take up and transport such drugs. Nanotechnology is a promising way in which this can be done:

NANOPARTICLES IN DIAGNOSTICS AND THERAPY

Nanoparticles can be used as a platform for therapeutic drugs; Dr Paras N. Prasad, distinguished professor of chemistry, Samuel P. Capen chair of chemistry, and director of the Institute for Lasers, Photonics, and Biophotonics (ILPB) at the University at Buffalo, together with a team of collaborators in May 2008 investigated nanoparticles on these carriers. In one such experiment rod-shaped semiconductor nanocrystals called quantum rods were linked to iron-transporting protein transferrin carriers in the capillaries of the brain which were allowed free access in and out the blood brain barrier. In doing this the blood brain barrier could be tricked into allowing the entry of the nanoparticles attached to the carriers. These quantum rods have a high surface area and can act as a platform to which therapeutic and diagnostic agents can be incorporated. These quantum rods were shown also to have low toxicity which would show them to be very suitable in treatment of neurological disorders. Quantum rods possessed the ability to emit electromagnetic radiation to a small change in their size so the transfer of co-delivered molecules carried by the rods could be monitored. Linking quantum rods to various different carriers and monitoring emissions of such radiations could be used to compare the efficiencies of different carriers in transporting substances.

The ability to emit radiation creates opportunities in diagnostics, and can be used as contrast agents in imaging with MRI (magnetic resonance imaging), the light emitted is much brighter than organic dyes and more easily stimulated; only one light source is needed. Thus very good images of tumour sites can be produced, higher contrast images at lower costs than today's current methods via organic dyes. But however, toxicity still needs to be controlled and monitored. Molecules that seek out and bind to tumours can be attached to the large surface area of these nanoparticles and due to their minute size allows them to accumulate preferentially at tumour sites.

A) Gadolinium is a malleable and rare ductile earth metal and traditionally, gadolinium-based contrast agents are used in MRI, which are injected intravenously and dye brain tumours by passing areas of disrupted blood brain barrier, which causes changes in magnetic resonance intensity by interaction with tumour tissue. However standard gadolinium-based agents solely image areas of tumour where blood brain barrier has been broken down and thus often fails to enhance the infiltrating tumour margins. Therefore there are areas of tumour that have not been enhanced and cannot be observed in gadolinium MRI imaging. Gadolinium has a relatively short half –life which is useful in that it can be quickly cleared from the body after use, but because of this property (and complex nature of tumours) gadolinium must be given at a specific time, during the course of an operation. Hence the gadolinium contrast agents may cause enhancement to areas of blood brain barrier that have due to surgical operation which are otherwise tumour free, and so issues arise in the distinguishing enhanced regions of brain tumour from areas of surgical disruption.

An alternative mode of imaging is nanocrystals of iron-oxide cores which may or may not be encapsulated within organic shells which affect movement and clearance of the nanoparticles within the body. Iron oxides are classic super magnetic contrast agents and are highly useful in magnetic resonance imaging. Studies have been carried out on several iron oxide based contrast agents and the low toxicity has been demonstrated, no clear evidence of

tissue damage or pathologic changes within the brain was observed on administered of the iron oxide nanoparticles.

Iron oxide-based contrast agents also interact with tumour tissue and cause enhancement of these areas by passing through regions of disrupted blood brain barrier in the same way as gadolinium-based agents, but display a range of differences once across the barrier. Iron oxides have the tendency to be taken up by reactive phagocyte cells, which are usually found at infiltrating tumour margins thus allowing detection of these tumour tissues that are not picked up by gadolinium

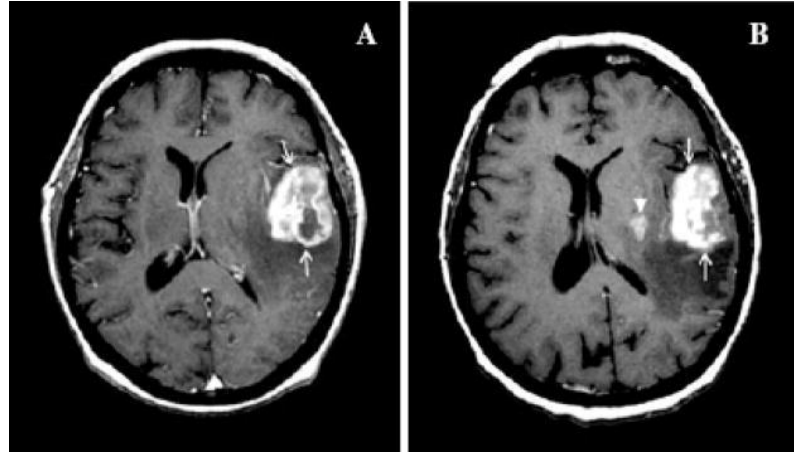


Figure 1: (A) Uses gadolinium based contrasting agent. (B) Iron oxide nanoparticles used as contrasting agent, which detects an area of enhancement close to the centre of the brain not picked up in 'A'

based contrast agents. This quality and the low diffusivity of the nanoparticles of iron

oxide, mean that these contrasting agents remain longer in these regions of tumour tissue rather than freely diffusing in surrounding tissues, and so giving a more accurate outline of the affected areas. Gadolinium on the contrary has a high diffusivity and so persists in these regions for a shorter time. These nanoparticles also cause the enhancement of tissues over a much longer period of time than that of gadolinium so it is possible to supply a dose 24 hours prior to surgery. The dosage does not cause enhancement to regions of surgical disruption, also unlike that of gadolinium, which is important when enhanced tissues detected in MRI are removed in surgery, so it is ensured that it is only the tumour tissues are removed.

B) The targeting of nanoparticle contrasting agents can be even further improved by coating the surface of iron oxide nanocrystals with tumour specific antibodies which proved effective in a study carried out on mice. Further methods included coating the nanoparticles in specific peptides or drugs which targeted tumour, the idea of targeting a specific section achieved high quality contrast enhancement images in recent studies.

The aim of steps such as the use of MRI is to identify specifically and clearly all areas of cancer growth in a field of view, for the possible diagnosis of brain tumours, and is the first step to combating the tumour. During surgery, marking areas of tumour in this way allows neurosurgeons to act so all and only the tumour tissue is completely removed, and other crucial structures surrounding the regions are not damaged. Doing so reduces the risk of recurrence of the cancer and lowering levels enough so that the remaining areas can be easily controlled and erased by complementary therapy which improves survival. Conversely, if this is not done then remaining residual tumours can lead to follow up treatment failure and the

need for additional surgery. Nanoparticles provide away of supplying fluorescent and visible dyes which can be delivered to stain tumour cells specifically and to an adequate quantity to achieve sufficient visual contrast for easy identification to a range of different tumours, intraoperatively for the removal of tumours as well as the diagnosis and identification of such.

Iron oxide based nanoparticles observed previously can also be loaded with molecules such as near-infrared fluorescing (NIRF) Cy5.5 which are coated with peptides such as chlorotoxin to improve tumour specific targeting. They can be taken up by the tumour cells of multiple types of cancers, producing highly accurate outlines of tumour margins for surgical removal. Their iron oxide core also allows the tumours to be observed under MRI. Quantum dots mentioned earlier may alternatively be used, improving their targeting by the attachment of tumour specific antibodies and using their ability of electromagnetic radiation emission to a change in size to induce fluorescent staining in brain tumours. Studies have shown both methods to be effective, but however, limitations of both methods include the requirement of sophisticated fluorescent imaging equipment technology. Darkened operative fields are also needed for illuminations of marked tumour areas to be seen, which would make the surgical removal of the tissues simultaneously impossible. The toxicity of quantum dots must also be watched due to their heavy metal content which may deem them unsuitable contrast agents.

New developments undergoing FDA (Food and Drug Administration) approval includes Nanocyan. Nanocyan is a nontoxic polyacrylamide nanoparticle loaded with high quantities of methylene-blue core and coated with tumour targeting peptides. This recent development is shown to give clear outlines of regions of tumour under normal operating lighting conditions and without the assistance of additional equipment. The high quantities of the methylene-blue dye allow tumour cells to be deeply stained and hence easily identified. Future use of Nanocyan shows huge potential in the ability to surgically remove brain tumours.

C) Other issues in the fight against tumours are to find an efficient means of therapeutic delivery such as that of delivering chemotherapy across the blood brain barrier. By assisting in passage across the barrier, nanoparticles can serve as not only methods to identify tumour tissues as observed previously but as platforms to enable the efficient delivery of drugs that would otherwise be unable to be delivered at effective therapeutic levels to brain tumours.

Examples of such applications are lipid-based drug delivery nanoparticles. Solid lipid nanoparticles (SLN) are a type of lipid-based nanoscale compounds developed for brain tumour drug delivery. They are derived from preparation of physiological lipids and so are nontoxic. SLN's are able to cross the blood brain barrier by the process of endocytosis due to circulating plasma proteins in the brain's blood vessels being taken up on the surface of the SLNs. This ability to cross the blood brain barrier raises huge potential in the delivery of drugs to the brain. The matrix structure of the SLN hence serves very useful as a means of

loading and encapsulating therapeutic drugs and protecting them from degrading. The composition of the SLN including its surface coating can be modified to control the unloading of drugs within targeted tumour tissues.

In recent studies, drug-loaded SLN's were shown to significantly increase drug concentrations within tumours subsequently decreasing concentrations within the blood plasma, achieving high drug bioavailability in tumour tissues in comparison to the equivalent doses of a freed drug. Higher drug bioavailability means recommended doses of the drug and hence any toxicity and side effects can be decreased. Nanoscale forms of low density lipoproteins are similar in composition to SLN's, maybe useful in the future drug delivery devices as the nanoparticles are preferentially taken up by tumour cells.

Liposomes are artificially prepared vesicles created by phospholipid bilayers that can encapsulate both hydrophobic and hydrophilic drugs. Nanoscale liposomes also show promise in the future treatment of brain tumours as they can rapidly enter sites of tumour tissue from the blood, but remain in the blood in the presence of healthy tissues and blood vessels. Previously explored developments of drug-loaded nanoliposomes were unsuccessful as poor distribution of the drugs within the tumour tissues resulted in low efficacy. However, improved delivery methods have offered opportunities in the redevelopment of nanoliposomes in the treatment of brain tumours. Topotecan has high systemic toxicity and so has not been used extensively in treatment but is considered part of chemotherapy in treating cancers. Recently developed liposomal topotecan via enhanced delivery methods were administered to animals with certain types of brain tumours (*glioma*) which enabled the delivery and accumulation high drug concentrations within the tumour, showing significant improvements in survival of the animals. The efficiency of liposomal drug delivery devices may be further enhanced through molecular targeting and thus can be regarded to have huge therapeutic potential.

D) The approach to combating tumours can be surgical, chemotherapeutic and via radiation treatment but there are concepts that are being developed for future use such as that of gene therapy. This is based on the idea where specific genes originating externally can be incorporated into the genome of a tumour cell to create a tumouricidal effect to inhibit the growth and prolong survival of the affected. Previous methods had included viruses and liposomes as vectors for delivering the gene therapy but these carried the risk of serious immune responses that may deem harmful and were inefficient. Nanoparticles have been recently developed as alternative non-viral vectors in which biopolymeric genes, encoded plasmids were incorporated into the nanoparticles were delivered and shown to be effective delaying growth of tumours by accumulating in tumour cells. The study proved successful in prolonging survival and inhibiting tumour growth in mice with certain types of brain tumour.

E) Further developments being explored included thermotherapy enabled by nanoparticles. This used the concept of inducing hyperthermia in cancerous tissues by injecting superparamagnetic iron oxide nanoparticles into tumours and heating them via an alternating magnetic field, which provided a way of easily and safely delivering heat deep into bodily structures. The heat inhibited the growth of tumour by decreasing the ability of tumour cells to divide, and showed prolonged survival in studies carried out on rats with specific brain tumours. These results allowed the trial of this thermotherapy on 14 patients, but however their response to the treatment was not as effective but there appeared to be no clear side effects or toxicity and so was deemed safe enough for further investigation so nanoparticle enabled thermotherapy may be improved and of great use in the future.

CONCLUSION

Nanotechnology clearly has huge potential for new and exciting advancements in diagnostics and therapies for combating brain tumours in the future. However the field is relatively young and so the long-term health effects, such as those regarding toxicity are unknown. The effects to health of ultrafine particles which are nanosized airborne products commonly present in natural and manmade pollutants can be observed to gain an idea of toxicity. Challenges simply in the engineering of nanoparticle pharmacology must be overcome, and factors such as clearing the nanoparticulates from the body must be considered. For example if it is excreted with faeces and bile as opposed to renally, the process of removal is slow, hence increasing the risk of long term toxicity from the remnants of nanoparticles; so we may try to develop nanoparticles for quick excretion with urine to overcome such issues. But as we carry out further investigations, and as our knowledge of the health effects and pharmacology expand in nanomedicine, these problems can be overcome and its potential in playing a vital role in the treatment and diagnosis of brain tumours in the future may be fulfilled.

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