

**NANOTECHNOLOGY, AND ITS POTENTIAL USES TO TREAT CANCER
IN THE CENTRAL NERVOUS SYSTEM (CNS) AS A METHOD OF
TRANSPORTING TREATMENTS ACROSS THE BLOOD BRAIN BARRIER
(BBB) WITH SPECIFIC REFERENCE TO NEUROBLASTOMA**

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ABSTRACT

Cancers such as Neuroblastoma often relapse in the Central Nervous system, beyond the Blood Brain Barrier, an area of the body which is inaccessible to any conventional cancer treatments. This paper discusses the potential of nanotechnology to act as a means of transporting drugs across the Blood Brain Barrier in order to allow treatment to take place, and in order to prevent relapse. This paper discusses: why cancer is an appropriate disease to be treated using nanotechnology? What sort of nanoparticle should be used to transport treatments across the blood brain barrier? And finally what type of treatment should the nanoparticle deliver to the cancer? This paper then goes on to discuss the theoretical possibility of using nanotechnology to transport protein based treatments, which would normally be too large, across the Blood Brain Barrier in small segments, and using the nanoparticles to assemble the full treatment, once across the Barrier, using complimentary base pairing. The paper concludes that the theory has merit, although there may be unforeseen side effects, and the question of how to remove the nanoparticles, once treatment has been administered, requires further investigation.

INTRODUCTION

“I want to build a million tiny factories, models of each other, which are manufacturing simultaneously ...manoeuvring things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; but has not been done because we are too big”

Richard Feynman, Nobel Prize winner in Physics- theorising the use of nanotechnology in 1959

The phrase “nanotechnology” refers to technology at the scale of one-billionth of a metre (referred to as nanometres) and it has countless potential industrial, technological and medical benefits. Nanotechnology can be described as the manipulation of systems at the molecular level; however, being a relatively new branch of science, very few of these uses have yet been exploited. Suggested as a theoretical science by Feynman (above), nanotechnology has recently become more common in day-to-day society. Obvious examples being in sun screens, where nanoparticles are used to make the substance less visible, and in glass windows, where nanoparticles prevent substances from sticking to the glass surface. When substances work at such small scales, their properties change dramatically (and this could be potentially advantageous). An obvious example of this is copper, which is opaque at the macro level but becomes transparent at Nano scales.¹ This alteration in properties is due to the dramatic change in surface area to volume ratio. Since the materials are so small and spread thinner, the surface area is suddenly far larger in comparison to the overall volume of the substance, and the quantum effects resulting from this are as such greater.¹ The staggering potential uses of nanotechnology could easily be, and indeed are, beginning to be applied to the field of medicine to enhance treatments, and it is this potential use of nanotechnology that I shall examine and discuss in this paper.

Different ways of applying nanotechnology to medicine (e.g. Cell repair, diagnostic and imaging techniques, and anti-microbial techniques⁹) are being researched, and one of the primary potential medical uses of nanotechnology is in drug delivery. A problem that has always arisen with drugs is the fact that, when they are injected or taken orally, they affect a vast proportion of the body, not only the area that needs treating, and the effect the drug can have on the rest of the body- known as the “side effects” of the drug- can often be negative.² A tactic known as “targeted treatment”, where the drug is only applied to the area of the body where it is necessary, is used to reduce the proportion of the body the drug affects, and as a result the chance of adverse side effects is reduced.² One potential way of doing this is using nanotechnology: the drug is encased in or attached to a nanoparticle and the contents are only released when the particle reaches the

appropriate cell. This would not only reduce the chance of side effects, it would also allow substances that are insoluble in the blood to be used as medicines. The nanoparticle could act as a protective layer, protecting the drug from “hydrolytic and enzymatic action in the gastrointestinal tract”.¹ Of course, there are potential problems with using nanotechnology in such a way. Firstly, how would the nanoparticle tell which cell to deliver the drug to? And secondly, why would nanotechnology, as opposed to another method, such as the use of liposomes, be the preferred method of targeted treatment? In this paper I shall discuss the potential uses of treating cancer, in a targeted manner, using nanotechnology.

The cancer I make specific reference to – Neuroblastoma, is a “neoplasm derived from the sympathetic nervous system [which is] a ...highly malignant, solid tumour” which manifests in children.⁶ This cancer often relapses in the brain, beyond what is called the Blood Brain Barrier (the BBB) which is composed of high-density cells, which restrict the passage of substances from the bloodstream much more than endothelial cells in capillaries elsewhere in the body, and this prevents toxins from entering the brain¹⁰. This represents a problem, because the CNS is a sanctuary site⁷(i.e. under normal circumstances, the cancer in the brain cannot be reached by conventional treatment) for Neuroblastoma, as standard treatments cannot cross the BBB (hence why the CNS is the site of relapse) I propose to solve this problem, and allow treatment to cross the BBB, using nanotechnology.

DISCUSSION

Why is Nanotechnology appropriate as a method of delivering drugs when treating cancer?

I have chosen to discuss the potential uses of nanotechnology in cancer treatment as I believe, due to the nature of the disease, the two dilemmas mentioned in the introduction can be solved when nanotechnology is applied to cancer as a method of drug delivery. The very fact that cancerous cells are different to the body’s healthy cells provides a potential means of differentiating between cells which need treating and cells which don’t. It is therefore possible that nanoparticles could be designed to distinguish between cells and only deliver treatment to appropriate ones, thus ensuring my first dilemma is solved. In addition, the treatments used for cancer would highly benefit from being delivered in a targeted manner, because many of them –chemotherapy, for example- have severe adverse effects on the patient when applied to their entire body (such as hair loss and sickness) and if only the cancerous cells were treated, then such problems would be solved.

As mentioned in the introduction, there are other vectors available besides nanoparticles, like liposomes, which could also be used to deliver drugs, and which vector is used in each circumstance depends to some extent, on the nature of the disease and the treatment. Therefore, in order to satisfy my second dilemma, I must show that, in terms of cancer (or at least, some specific cancers, such as Neuroblastoma) Nanotechnology is the logical method of drug delivery.

Perhaps the most obvious advantage of nanoparticles as a method of targeted treatment is their size; the fact that they are so incredibly small means they can be transported to areas of the body which are inaccessible to other substances which are used as methods of delivering drugs. This means we have a valid reason why nanoparticles should be the vector of choice. An obvious example

of an area in the body which is usually inaccessible to drugs and other forms of targeted drug treatments is the central nervous system, because of the blood-brain barrier, which “represents a formidable obstacle, preventing drugs from penetrating into the central nervous system”⁴. Crossing the blood-brain barrier is of vital importance in treating cancer because about 1 in 4 patients with cancer will develop tumours that spread to the central nervous system⁵ and so an effective method of treating this problem is urgently required. Neuroblastoma is particularly relevant to my theoretical treatment because “in 64% of patients the CNS is the sole site of recurrence” and so the BBB represents an obstacle for treatments⁷. I propose that it would be possible to overcome this obstacle by using nanotechnology.

Tests have already confirmed that Neuroblastoma would be appropriate for treatment using nanotechnology, as Neuroblastoma cells possess a carbohydrate antigen known as GD2, which is not present in normal tissues, and so it is “an attractive ligand for Neuroblastoma therapy”⁶, and could be used by nanoparticles to identify cancerous cells. Indeed this process has been tested, and “carbon nanotubes were functionalised with monoclonal antibodies to specifically target Neuroblastoma cells with GD2 on the cell surface”⁶. Through this research, it was concluded that this method had “high potential” in treating Neuroblastoma⁶, as nanoparticles would provide a means to locate cancer cells. Nanotechnology is an appropriate method of drug delivery because it is potentially possible for the nanoparticles to be altered so they can differentiate between cancerous and healthy cells; and, since nanoparticles are small enough to cross the BBB, they are the most logical choice of vector for delivering drugs to cancer on the other side of the barrier.

What sort of nanoparticle would be appropriate to deliver the cancer treatment across the BBB?

Research into the use of targeted drug delivery to treat cancer has been undertaken by researchers in the University of Michigan’s centre for Biologic Nanotechnology. They refer to the type of nanoparticle they use as a “dendrimer,” and these particles have the advantage of having what Almut Mecke (a physics doctoral student who worked on the project) described as “loose ends”³. These could accommodate additional attachments -such as targeting agents- which could distinguish cancer cells from healthy cells³, and therefore could potentially be effective at locating cancer cells. The feature of being able to attach substances to this particular nanoparticle means that it may be possible to attach a drug to the outside of the particle that has the capacity to kill cancerous cells (or treat the cancer in some other way).

Further additions could also be applied to the outside of these nanoparticles, such as biosensors, which could report how effective a particular treatment has been.³ From their data I can conclude that, in any future proposed use of nanotechnology to treat cancer, the dendrimer is a valid choice.

I am provided with further cause for optimism by a review article regarding nanotechnology and drug delivery by Nelson A Ochekepe, Patrick O Olorunfemi, and Ndidi C Ngwuluka, which states that it is possible to use nanoparticles to penetrate the blood brain barrier¹, (as I hypothesised) and therefore I believe it is plausible that nanoparticles such as those used Michigan University could travel through the blood brain barrier carrying both cancer cell detectors and treatments (providing the additions to the nanoparticles were not large enough to prevent the crossing of the blood-brain barrier).

What treatment/treatments should be carried across the BBB by the Nanoparticles?

[PLEASE NOTE: from this point onwards, any conclusions drawn, whilst grounded in existing science, are highly theoretical, and so, even though they have been reached through logical reasoning, experimental testing would be required to fully validate any ideas put forward]

The question of what sort of treatment that nanoparticles would deliver to the cancer cells once across the BBB has not yet been addressed, and so I shall now consider potential drug based cancer treatments and the advantages/disadvantages of using them in this manner.

Chemotherapy

Perhaps the most well-known – and infamous – drug based cancer treatment is chemotherapy, which aims to kill cancer cells by killing all types of cells which divide rapidly. A drug used in chemotherapy could be attached to one of the “loose ends” of the dendrimer (as could a targeting agent, which could be used to locate the GD2 antigens on the Neuroblastoma cells) and the dendrimer/drug/targeting agent complex could then seek out and treat the cancer cells exclusively.

Since the chemotherapy drugs do not distinguish between healthy dividing cells (such as hair follicle cells) and cancer cells, chemotherapy has the side effect of being damaging to patients, and therefore it may be advantageous to use targeted treatment in this way in very many types of cancer in all areas of the body (not just cancer beyond the BBB) as this may reduce the chance of side effects such as hair loss. However, in the specific case of cancer beyond the BBB, this may not be an appropriate course of action, because the majority of chemotherapy drugs are unable to pass through the BBB, and so, even when attached to a nanoparticle designed to seek out cancer cells, the drugs would be too large to cross the barrier and treat the cancer.

Antiangiogenic agents

Drugs known as antiangiogenic agents are another class of drugs used to treat cancer, and could potentially be attached to the nanoparticles and delivered into the CNS through the BBB. These drugs help to prevent new blood vessels, which would provide the tumour with nutrients, from forming. The process of blood vessel formation in this manner is known as angiogenesis. Unfortunately, it is also very possible that some of these drugs are, like the drugs used in chemotherapy, unable to penetrate the BBB. However, antiangiogenic agents can sometimes be proteins or peptides, and therefore I believe a possible solution could be to attach their constituent amino acids separately to individual nanoparticles, and have them assemble into the full drug once across the barrier. This seems plausible because systems exist that transport amino acids from the Bloodstream to the Brain¹². Every different amino acid necessary to create the desired protein (in this case, the antiangiogenic drug) would be attached, in the correct quantity, and, once on the other side of the blood brain barrier, these amino acids could assemble into the larger protein antiangiogenic drug. The nanoparticles would also need to have a feature which allowed the different nanoparticles to align in the correct order, so that the amino acids were in the correct sequence. Since the process I am describing is so similar to the translation process that occurs in cells during protein synthesis, I see no reason why the same bases (Adenine and Thymine, along with Guanine, and Adenine) should not be used to determine this order through the use of

complimentary base pairing. This would work by attaching a particular combination of bases to a certain nanoparticle, (along, of course, with a specific amino acid, which will always correspond to the particular combination of bases chosen) and, the nanoparticle attached to the next amino acid in the sequence would have to also be attached to the necessary complimentary base pairs to ensure the amino acid was placed at the correct point in the sequence. It is possible that a certain amino acid may appear at two or more different points on the protein's chain, and of course, it is therefore possible that the same amino acid may be bonded to a nanoparticle with one combination of bases as well as being bonded to another nanoparticle with different bases, which would have to be done in order to ensure the amino acid in question features at all required points along the chain. The final amino acid in the chain would have to feature some sort of "stopping code" as opposed to more bases, to ensure the synthesis stopped when necessary. The process may have to take place within cells on the other side of the blood Brain Barrier, so the ribosomes within the cell can be utilised in the synthesis process (in the same way that a virus invades and utilises the contents of a cell). The amino acids would have to be bonded to the nanoparticle in such a way that when the peptide bond formed, the bond between the nanoparticle and the amino acid broke.

Antibodies

One problem with using antiangiogenic drugs is that they do not attack the cancer cells in particular, and this is a problem because a key issue with the idea I have suggested is that the nanoparticles need to group together somehow on the other side of the BBB. One possible way of achieving this would be for them to assemble at the site, or even inside, of the cancer cell, because the nanoparticles can locate the tumours. This would be one obvious way of ensuring that the nanoparticles grouped together, thus allowing the amino acids to combine, on the other side of the BBB. If we were to take this approach, we would have to use another form of treatment (which is also a protein, and therefore made up of amino acids) which attacked the cancer cells in particular, and an obvious option would be antibodies (an appropriate choice, because antibodies are starting to be used in the USA as a treatment for Neuroblastoma⁵). They are a particularly logical choice because, they improve survival among children with high risk Neuroblastoma (as discussed in a paper published in September 2010 in the New England Journal of Medicine)¹¹; and, as Kushner states in his paper, "monoclonal antibodies [an antibody used in the treatment of Neuroblastoma] may have improved survival but do not penetrate the BBB"⁷. A potential remedy to this problem would be to use my theoretical idea and introduce the monoclonal antibodies into the CNS in small building blocks and have them assemble on the other side using complimentary base pairing. The building blocks of the antibody would pass across the BBB, each attached to a particular nanoparticle, and the nanoparticle would also be modified so that it located cancerous cells. Once at the site of the tumour, the building blocks would assemble into the antibody due to the positioning of base pairs on each nanoparticle. In order to increase the likelihood that enough antibodies form to make a significant difference, a concentrated dose would have to be used, as this would increase the probability that a high number of antibodies would form at each cancerous cell.

Of course, another way to get these antibodies across the Blood Brain Barrier into the CNS would be to use a lumbar puncture⁸, but this is incredibly painful, and since Neuroblastoma occurs in children, the less painful solution of injecting the antibody in small protein building blocks would be preferable. In addition, when antibodies are injected, they can damage healthy brain cells as well, potentially causing brain damage⁸. However, if the antibodies are assembled at the site of the cancer

cells, the chance of the antibodies coming into contact with healthy cells is greatly reduced, and so when the tactic of targeted treatment is adopted, I suspect that the adverse side effects of antibody treatment will be greatly reduced, because fewer healthy cells will come into contact with the antibodies.

SUMMARY AND CONCLUSION

It is my belief that the treatment of Cancer, and in particular Neuroblastoma, will become more effective as a result of the use of nanotechnology as a form of targeted drug delivery because it will allow treatment to reach the cancer located across the BBB. Nanotechnology will also reduce the proportion of the body's cells which are affected by the treatment, and this could greatly reduce the adverse side effects of some of the more potent cancer treatments, such as chemotherapy. Since cancer cells are different in makeup to normal body cells, nanoparticles (such as the University of Michigan's "dendrimer", which I believe is a logical nanoparticle to use to deliver cancer treatment) could easily be modified to distinguish between the cells which need treatment and the cells which don't, making nanotechnology an effective method of targeted treatment. Since, in many cancers, such as Neuroblastoma, the site of recurrence of the cancer is often beyond the BBB (blood-brain barrier), treatment is often unable to reach the cancer as it is unable to cross this barrier. Nanotechnology is extremely useful on this front because nanoparticles are small enough to cross the BBB, and can carry treatments, such as antibodies, across the BBB. By attaching protein based treatments, such as antibodies, to the nanoparticle, the nanoparticle may be made too big to cross the BBB. A more logical course of action may therefore be for each nanoparticle to take an individual amino acid across the BBB, and the antibody protein could be assembled on the other side of the BBB, at the site of the cancer cell, using a system similar to complimentary base pairing. Antibodies are the most appropriate choice of treatment to use when treating cancer in this way, because they work by attacking the cancer cells specifically (and so, unlike antiangiogenic agents, they would benefit from being attached to a nanoparticle which possessed a targeting agent designed to locate cancer cells). In addition, antibodies, unlike chemotherapy drugs, are protein based, and so could be assembled on the far side of the BBB once the smaller, more transportable building block amino acids had been carried across the BBB attached to individual nanoparticles.

An obvious potential disadvantage of preparing nanoparticles in this way is the possible cost of such a treatment. However, I believe that, in the future, as the use of nanotechnology in the medical field becomes more common, and as our ability to synthesise such particles improves, the cost of nanotechnology will be greatly reduced and this will no pose a significant issue. Perhaps a more significant problem my theoretical idea presents is the issue of how to remove the nanoparticles once they have delivered the treatment. This could be done by ensuring that they were structured in such a way that once they had released the amino acids, the state in which they are left makes them easily hydrolysable, or perhaps they could be engineered to decay into harmless substances over time.

A problem with any theoretical treatment is that the full effects of the treatment on the patient (including potential unwanted side effects) can never be fully known until reliable testing takes place – and even then some rare side effects may remain unknown. Given that I have been speaking purely theoretically, I fully acknowledge the possibility that there may be some unforeseen negative consequence of my proposed action and so rigorous lab and drug testing would be required before

treatment begun, in order to ensure we are made aware of any unwanted side effects of treating cancer in this way.

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