MEDICAL APPLICATIONS OF NANOTECHNOLOGY – THE FUTURE ROLE OF NANOTECHNOLOGY IN DETECTING AND TREATING CANCER

BY

MELISSA HARTLEY

PASS WITH MERIT

RESEARCH PAPER

BASED ON

PATHOLOGY LECTURES

AT MEDLINK 2010

ABSTRACT

Nanotechnology has many implications within the medical field, ranging from the production of materials to increase drug bioavailability to the use of magnetic nanoparticles to stimulate nerve regeneration. This paper discusses the potential of nanotechnology with regards to cancer therapies and diagnostics, as well as the ethical issues relating to the use of relatively untested, experimental technologies and treatments. Treatments utilising nanoparticles inserted into the affected cells alongside radiotherapy show the potential to treat the disease non-invasively and without damage to surrounding tissue, while quantum dots may allow detection before the cancer has progressed to a stage where treatment is not only difficult, but lengthy and plagued with side effects. However, the possible toxicity of certain types of nanoparticles could lead to new and conceivably lethal secondary effects, so further research is required in order to learn which nanoparticles could be used in the treatments currently being tested.

INTRODUCTION

Cancer is one of the leading causes of death worldwide, accounting for around 13% of all deaths in 2004. More than 1 in 3 people in the UK contract the disease during the course of their lives, and the prevalence of the disease is expected to continue rising due in part to the aging population. Each year, Cancer Research UK alone spends approximately £355 million on researching potential new treatments for one of the most widespread, yet least curable diseases of current times. Existing cancer therapies are often ineffective as well as leading to multiple side effects and damage to non-malignant tissue. A substantial proportion of current research is focused on nanotechnology and the various ways in which it could assist in the detection, management and even cure of cancer. Nanotechnology refers to the control and manipulation of matter on an atomic or molecular scale ($1nm=1x10^{-9}m$), allowing the synthesis of materials with a range of properties, many of which are unobtainable using traditional materials. Currently, advances are being made with regards to the use of nanoparticles as drug carriers to allow targeted drug delivery, therefore increasing bioavailability (the fraction of administered drug available for use in the targeted tissue). This research is not specific to chemotherapeutic drugs, but has the potential to play a large role in future cancer treatments, cutting down on both unwanted side effects and costs, as a smaller fraction of the drug would be wasted.

The use of nanoparticles to increase the responsiveness of cancerous cells to radiotherapy has also been investigated to an extent. One 2005 study conducted by Dr Hongjie Dai and his team of researchers at Stanford University investigated the use of carbon nanotubes and radiation in destroying cancerous cells. First discovered in 1991, carbon nanotubes are allotropes of carbon with a cylindrical structure. Single-walled nanotubes (SWNTs) are used in this form of cancer treatment. The tubes have a diameter of only a few nanometres, so thousands could fit inside a typical cell. Like other nanomaterials, these tubes have unique properties which make them suitable for use in medicine. Nanotubes are efficient thermal conductors, and so can easily be heated by radiation once inside the targeted cells, causing thermal destruction of the malignant tissue. In this study, SWNTs were inserted into only the cancerous cells, and upon exposure to near-infrared light from a laser they heated up, killing only the targeted cells. However, other research has suggested that the SWNTs used in these experiments are highly toxic to the human body, meaning that, while the principal behind the treatment is sound, currently it may cause more harm than benefit. This highlights a major obstacle that needs to be overcome when developing nanotechnology-based treatments, and one aim of this paper is to look at the effects of different nanoparticles on the body for use both in treatments and diagnostics.

DISCUSSION

Developments in nanotechnology could lead to new imaging techniques able to detect the disease at an earlier stage than is possible at present. Current cancer detection methods require the disease to have progressed to a stage where a visible change has been made to the affected tissue. In order to minimise damage cause by the disease and improve long term prognosis, it is vital to detect and treat the cancer as early as possible. Nanotechnology may be able to provide methods of detecting cancerous and potentially even pre-cancerous cells due to the unique electrical properties of certain nanoparticles. One method by which this is possible is through a new DNA test involving the use of quantum dots, nanoparticles of semiconductor material 2-10nm in size. Researchers at the Johns Hopkins Kimmel Cancer Center have discovered that, due to the ability of the quantum dots to easily transfer energy, they can be used to make cancer related strands of DNA light up, enabling them to easily be noticed and counted.

The test works by using quantum dots to detect abnormal DNA methylation (when a methyl group attaches to cytosine, a DNA nucleotide). While methylation can help suppress harmful genetic information, it also plays a vital role in the development of cancer as it can prevent the release of tumour-suppressing proteins. As a result, abnormal DNA methylation is considered to be indicative that cancer is developing or is likely to develop. In this test, the quantum dots were coated with a chemical which is attracted to a molecule on copies of target DNA strands. Upon exposure to UV light, the quantum dots transfer the energy to fluorescent dyes attached to the DNA strands, causing them to light up. Using a spectrophotometer, the researchers were then able to analyse the signals and detect the presence, and quantity, of DNA methylation.

Provided that future research shows that this test could safely and viably be reproduced on a wide scale, this would be a major breakthrough, allowing much earlier detection of cancer than at present. It is possible that quantum dots may also be of use in the detection of diseases other than cancer, if different chemicals were used to bind to early signs of the disease in question. If this technology could be integrated into a cancer screening programme, targeting in particular those demographic groups most at risk of the disease, then the rate of undetected cancer could be dramatically reduced. Additionally, as this test provides data about the quantity of the cancer-linked DNA, doctors would be able to determine how far the cancer had progressed and treat accordingly. This would be a significant improvement on current screening methods, which often only detect cancer at a late stage. Due to the limitations of existing cancer treatments, the cancer can then only be treated generically, and this is often too little, too late, or ineffective in individual circumstances. This highlights the need for not only improved screening methods, but also new treatments which are able to treat later-stage cancers more efficiently.

Nanoparticles are small enough to enter cells without damage, and the research I have found has led me to believe that this technology could be the key to new, more effective cancer therapies. Whether this is by inserting nanoparticles into cancerous cells and using these particles to destroy malignant tissue, or by using nanocarriers to deliver drugs directly to affected cells, the damage to other cells would be minimal. However, this is dependent on the discovery of nanoparticles which are tolerated by the human body. The physical and chemical properties of nanoparticles vary greatly from those of large scale materials even when made from the same substance, and as a result the effects on the body may be different. Although their small size makes the particles ideal for targeting and entering specific cells, should the body detect them as foreign particles an immune response would be triggered, potentially leading to inflammation and irritation. The risk of discovery and destruction of the particles by the immune system could possibly be overcome by coating them with polyethylene glycol – a polymer already used in gene therapies to shield viral vectors from the immune system – allowing them to reach the target cells without being destroyed. This could be vital when using nanocarriers to deliver drugs directly to cells if the particles are to arrive undamaged, and could form the basis for a new, more effective form of chemotherapy.

Administering chemotherapeutic drugs without damaging healthy tissue has proved to be one of the major obstacles in cancer therapies. Most antineoplastic drugs work by impairing mitosis, thus preventing the division of cancerous cells. However, current methods of administration are non specific and therefore drugs also target non-cancerous, but rapidly dividing cells. If a way to target only cancerous cells could be found, then the negative impact on healthy tissue could be significantly reduced. Some of the latest research in this area, published in July 2010, has found a way to form nanoparticles from a currently used cancer drug, with the size of the particles reducing the side effects associated with the drug. Cisplatin is one of the first drugs given to cancer patients, and has been used since the 1970s. However, it is known to cause kidney damage, limiting the dose physicians are able to administer and therefore the effectiveness of the drug. Scientists at the Harvard-MIT Division of Health Sciences and Technology have developed cisplatin nanoparticles (sized 100nm) which are too large to be absorbed by kidneys, yet are able to reach tumours due to the 500nm pores in the surrounding blood vessels. This allows higher doses of the drug to be dispensed without fear of causing damage to kidneys, and so may significantly improve efficiency of the drug and reduce treatment times.

Another group of cancer drugs, which have yet to be approved, are the CDK (Cyclin-dependent kinase) inhibitors. CDKs are responsible for regulating the cell cycle, and by preventing their action in cancerous cells, the division of such cells could be prevented. However, the damage would be severe if cells other than those intended were affected. If these inhibitors could be made into nano-sized particles able to reach only cancerous cells, these drugs could become an alternative to cisplatin. This would be particularly useful for patients who are cisplatin-intolerant. It may be possible to apply this technology to other drugs to treat a variety of diseases, but it is particularly suited to cancer due to the numerous side effects associated with current treatments.

Another way cancerous cells could be targeted is through the use of specific drugs, shaped to bind only to receptors on the surface of targeted cells, opening up protein channels and allowing nanoparticles to enter, and subsequently destroy, the cancerous cells. Dr Hongjie Dai's study, detailed in the introduction, is based on a similar idea; however there is debate over the safety of the carbon nanotubes used in his study. Researchers at the University of Texas Medical School and NASA Johnson Space Center have shown that upon inhalation, carbon nanotubes may cause widespread necrosis and even death. The toxicity of these nanotubes was investigated by introducing the fibres into the trachea of mice. The mice were killed either 7 or 90 days after treatment in order to allow examination of the lungs. However, while mice treated with a low dose (0.1mg) of nickel-yttrium containing nanotubes showed no adverse effects, 5 of 9 mice treated with a high dose (0.5mg) died within the first week, suggesting a far higher risk posed by the nanotubes than initially expected. Upon examination under a microscope, the lungs of these mice showed severe inflammation and granulomas. It is unclear whether the deaths of the five mice were caused by the nanotubes themselves or by the nickel and yttrium in the nanotube sample. Given that the deaths did not occur with the other nanotubes samples ('raw' and 'purified') it is possible that nanotubes could be relatively safe or lethal, depending on the methods used in the production of the tubes. However, as the nanotubes were only tested on mice, the only effect which has been documented is the effect of the tubes on mice. Before conclusions about the suitability of the particles for medical use could be drawn, tests would have to be carried out on humans. While a significant difference between the results is unlikely, it is possible that variation between the immune system and tissues of rats and those of humans may lead to one species tolerating the particles where the other may not.

Conversely, other studies into this area have suggested that carbon nanotubes pose little to no threat to human health. Scientists from the Institute of Toxicology and Genetics at the Karlsruhe Research Center in Karlsruhe, Germany have produced results which could explain why previous tests had shown SWNTs to be highly toxic. In their experiments, human lung cells were exposed to the SWNTs, and the toxicity of the tubes gauged in four ways. Three of the tests showed no cytotoxicity, while one, the MTT assay, appeared to produce a 'false positive', indicating that previous studies may have wrongly concluded that SWNTs were toxic to the body. The MTT assay measures how the salt MTT (methylthiazol tetrazolium) is chemically converted to a purple dye (formazan), a process which will only take place if mitochnodria enzymes in the cell are active. When this test was carried out on cells previously exposed to the nanotubes, the salt was not converted to dye, indicating that the cells had been damaged by the SWNTs. Upon observation using an electron microscope, the researchers concluded that the anomalous result was due to the non-soluble nature of MTT and formazan, as this led to the MTT-formazan crystals covering and reacting with the nanotubes. As a result the formazan was made undetectable and the nanotubes appeared to have poisoned the cell. This study, while providing evidence that carbon nanotubes may after all be safe for use in treatments, only highlights the need for extensive testing to ensure that these particles will not cause more harm than benefit. If the studies suggesting carbon nanotubes are toxic produced accurate results then working with nanotubes could pose a significant risk, both to doctors administering the treatment and to patients. Further research is required to determine the extent of the toxicity of the nanotubes, if they are indeed dangerous at all, before they could be considered for use in clinical trials and eventually in widely available treatments. If they cannot be proven to be safe, then the medical use of carbon nanotubes would be restricted to those procedures and techniques which do not involve introducing the particles to the body, and even then, precautions must be taken to ensure the safety of those carrying out the procedures.

Other studies have investigated the use of alternative nanoparticles. One similar method of cancer therapy, Kanzius RF therapy, is currently in the experimental stages, with clinical trials due to start in 2012. The therapy uses gold nanoparticles (GNPs) of diameter 5nm coated with cancer-seeking molecules, which seek out and bind only to cancerous cells. Upon exposure to radiation, the nanoparticles heat up and destroy the malignant cells. The antibody cetuximab has been used to detect and target cancerous cells, and the rate of intracellular uptake has been shown to be significantly improved by its presence. Almost 100% cytotoxicity was produced in cells treated with cetuximab-covered gold nanoparticles, while the rate of cytotoxicity in the control groups was significantly lower. GNPs are currently used for this treatment as alone they are tolerated by the human body, producing no cytotoxicity. However, when combined with radiation, thermal destruction of the malignant cells occurs. In these experiments, conducted by researchers at the University of Texas and The Department of Biomedical Engineering, Mayo Clinic, Rochester, Minnesota and published in 2008, cytotoxicity in cells treated with a high dose of GNPs ranged from 96.5% to 99.8%, depending on the type of cell and the length of exposure to radiation - either 1,2 or 5 minutes. Cells treated with lower doses of GNPs showed lower rates of cytotoxicity, as did cells exposed to radiation in the absence of GNPs. These results, particularly the findings that gold nanoparticles pose no threat to the body, suggest that cancer treatments such as this could become a reality in the foreseeable future, provided the nanoparticles and radiation can be administered in such a way that doesn't damage non-malignant tissue surrounding the targeted areas.

When using radiation, it is important that the most effective, yet least ionising and therefore least dangerous frequency possible is used. An additional shortcoming of the method highlighted in the introduction is that the near-infrared light will only penetrate the body to a depth of 2-3cm, so it would only be effective when dealing with superficial malignant tumours. However, it may be possible access deeper tumours through surgical methods, and then make use of nanoparticles and radiation. This should still damage less healthy tissue than removing the entire tumour via surgery. Further studies should focus on finding the optimum frequency of radiation to cause maximum impact with minimal damage and/or finding particles which are responsive to the lowest possible dose of radiation. The effects of different sized nanoparticles within the nanoscale range should also be investigated in order to determine how using many smaller particles or fewer larger particles would affect the efficiency of the treatment, in order to allow further minimisation of the amount of radiation necessary.

ETHICAL CONSIDERATIONS

In order to gain a perspective on how ethical the use of nanotechnology-based treatments is, they must be compared to the ethical issues with regards to current treatments. In these treatments, healthy cells are often destroyed as a secondary effect to the destruction of malignant tissue. One of the principles of medical ethics is nonmaleficience, meaning "to abstain from doing harm". In accordance with this principle, physicians must refrain from providing treatments which are ineffective, or where the negative effects outweigh the beneficial effects. The possible side effects which could be caused by nanotechnology based treatments must be weighed against the benefits that the treatment will provide. Given the severity of cancer, it is unlikely that harm from these treatments will be greater than the benefit to the patient in the majority of cases, provided that they are tested to sufficiently high standards. Additionally, one of the major problems with current cancer treatments is the severity of the side effects, therefore for new treatments to be more ethical they would only have to cause less harm than those used at present.

The possibility of unexpected side effects occurring must also be considered when dealing with new treatments, as despite thorough clinical testing, the risk of new side effects presenting themselves mustn't be ignored. Another factor to consider is that clinical trials may not show the long term effects of a treatment – the onset of symptoms may occur several years afterwards. Thus it could be considered unethical and a breach of the Hippocratic Oath sworn by physicians to provide patients with treatments which could potentially cause more harm than good. However, this risk is not unique to nanotechnology based treatments, but is shared by any new medication or therapy and as such cannot reasonably be used as a basis to declare the treatment unethical.

Furthermore, the morality of testing new treatments, in this case nanoparticles, on animals must be considered. In the study above, all mice given doses of carbon nanotubes either died or were killed, after possibly suffering extensively. While the testing of cosmetics and other non-essential products on animals is clearly immoral, the issue of the animal testing of medicines is less clear-cut. Many of the treatments tested will go on to save many lives, and often can be used on animals. Similarly, it can be argued that if the death/injury of a small group of animals prevents a treatment from going into production and harming thousands of humans it is surely 'the lesser of two evils'. One of the major issues with animal testing is that the subjects are unable to give consent, whereas in human drug trials all participants are volunteers aware of potential risks. Additionally, ninety-two per cent of potential human drugs that pass preclinical testing (including animal tests) are rejected during human clinical trials. Results produced in animals may not accurately reflect human reactions to a drug or treatment, so both human and animal test subjects suffer from the experimentation. The best solution is a compromise allowing animal testing, but only as a last resort. Alternatives such as in vitro testing and computer models can provide results accurate enough that in many cases the animal testing stage may be eliminated altogether. In the case of nanoparticles, clinical trials must be carried out before they can be used in medicines, and as animals may react differently to humans exposed to the same particles, the necessity of animal testing is questionable. It is therefore only ethical to continue experimentation on animals if no alternatives are available.

CONCLUSIONS

Nanotechnology has the potential to revolutionise the way diseases such as cancer are detected and treated - a combination of DNA screening utilising quantum dots and improved treatments could significantly increase the likelihood of recovery from the disease. Current drugs may be modified in the same way as cisplatin in order to manipulate their properties and reduce side effects. However, while treatments such as Kanzius RF therapy are promising new developments, currently they are only in the preliminary stages of testing. While the technology is available, much more research is needed to establish the safest and most ethical method of treatment, as if the technology is to improve upon the traditional methods of cancer therapy – primarily chemotherapy and radiotherapy – the side effects must be minimal. Due to the potentially hazardous nature of certain types of nanoparticles, tests must be carried out in order to determine which types of particles would be most suitable for treatment. Additionally, many of the studies done to this point have only tested the effect of the particles on animals or tissues. While it is necessary to test potential drugs and treatments before they can be used on humans, there is debate surrounding the necessity of animal testing due to its apparent amorality. However, as any treatments developed through this method of testing may also be available for future use on animals, the benefits are likely to outweigh the disadvantages given that treatments are tested for adverse effects on tissues before being administered to live animals. Nonetheless, due to variation between animals and between animals and humans, until the particles are tested on live humans, their effects, and therefore suitability for use in cancer treatments, cannot be known for certain. Although the studies highlighted in this paper, among others, have gone some way into discovering the safety of the nanoparticles intended for use in these therapies, in order to justify the further development and potential use of such treatments they must be proven to be safer than current therapies.

SOURCES

Alternatives to animal testing
http://www.andrewknight.info/publications/anim_expts_overall/summaries/AK%20Anim%20expts%20Vet%20Times%202009%2039%2845%29%208,10.pdf

Benefits for Diagnosis http://nano.cancer.gov/learn/impact/diagnosis.asp

Benefits for Treatment and Clinical Outcomes
http://nano.cancer.gov/learn/impact/treatment.asp

Cancer Fact sheet N°297 http://www.who.int/mediacentre/factsheets/fs297/en/index.html

Cancer Therapy Without Side Effects Nearing Trials
http://www.wired.com/medtech/health/news/2008/04/kanzius_therapy

Carbon-Nanotube Toxicity Test Tricks Scientists
http://www.physorg.com/news76672397.html

CDK inhibitors in clinical development for the treatment of cancer
http://informahealthcare.com/doi/abs/10.1517/13543784.12.6.955?journalCode=eid

Hongjie Dai's research lab at Stanford University
http://www.stanford.edu/dept/chemistry/faculty/dai/group/

Improving Cisplatin with Nanoparticles
http://nano.cancer.gov/action/news/2010/aug/nanotech_news_2010-08-17e.asp

Intracellular gold nanoparticles enhance non-invasive radiofrequency thermal destruction of human gastrointestinal cancer cells
HTTP://WWW.JNANOBIOTECHNOLOGY.COM/CONTENT/6/1/2

Nanotechnology kills cancer cells http://news.bbc.co.uk/1/hi/health/4734507.stm

Nanotubes Highly Toxic http://www.i-sis.org.uk/nanotubestoxic.php

New DNA test uses nanotechnology to find early signs of cancer
http://www.physorg.com/news169727891.html

Non-Invasive Radiofrequency Ablation of Cancer Targeted by Gold Nanoparticles
http://www.surgjournal.com/article/S0039-6060%2808%2900252-3/abstract

Noninvasive radiofrequency field-induced hyperthermic cytotoxicity in human cancer cells using cetuximab-targeted gold nanoparticles.
http://www.ncbi.nlm.nih.gov/pubmed/19227011

Polyethylene glycol http://www.mahalo.com/polyethylene-glycol/

Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation http://www.rmaoem.org/Pdf%20docs/Nanotubes-pulm%20toxicity.pdf

Statistics on the risk of developing cancer
http://info.cancerresearchuk.org/cancerstats/incidence/risk/

What Is DNA Methylation? http://www.wisegeek.com/what-is-dna-methylation.htm

Working with Government
http://info.cancerresearchuk.org/publicpolicy/workingwithgovernment/