

**Using Modified C60 Fullerene Nanoparticles in the Specific
Treatment of Cancer as Opposed to Non-specific Chemotherapy**

By

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Abstract

Chemotherapy is a very commonplace standard practice for the treatment of aggressive or metastatic cancers. It can take advantage of the extremely high metabolism of tumours to kill them off far faster than surrounding benign tissue. But the amount of what is, in effect, poison that has to be injected into the patient can cause very severe side effects.

We, here, suggest an alternative involving the innovative new Buckyball based drug delivery systems to take the treatment chemicals to the tumours and only the tumours, in order to limit the damage to the surrounding tissue and the harm caused to the patient in the process, whilst retaining the effectiveness of chemotherapy. This paper finds that the treatment could prove hugely useful in the future, as it allows us to vastly increase the effectiveness of our treatments against the more difficult cancers.

Introduction

Small Cell Lung Carcinoma (SCLC) is a cancer of the lungs with a very high metastasizing potential. Tumours have been known to navigate from the blood into the lymph, the bone marrow, the blood and the brain. The effect of the cancer itself is exacerbated by the fact that it secretes Vasopressin^[1] (an anti-diuretic hormone) which can lead to fluid overload in severe cases. This can lead to heart failure or paroxysmal nocturnal dyspnea, which serves to enhance the effects of the cancer. The outlook for patients with this cancer is extremely bleak; 65-70% of patients are presenting disseminated or extensive stage cancer at the time of diagnosis^[2]. At the extensive stage, the disease is incurable, and the median survival time is about 6 weeks^[2]. For patients who are not yet at the extensive stage, a course of chemotherapy can help. However, as mentioned above, due to the non-specific nature of chemotherapy it can cause more damage than good in some cases, occasionally even leading

to the deaths of older or weaker patients. The specific chemotherapy medicines used in treating SCLC include cyclophosphamide and cisplatin. Cyclophosphamide has been shown to contribute to cancer of the bladder in the long term, which will require further chemotherapy or surgery to repair, whilst cisplatin has been connected to nephrotoxicity, neurotoxicity, deafness, electrolyte disturbance and severe nausea/vomiting. At this point, several of these side-effects cannot be prevented or treated, and the cost involved in repairing the damage of those that can be treated (such as

cancer of the bladder) vastly increase the expense involved in treatment, with a course of Cisplatin and Paclitaxel (mitotic inhibitor almost always administered with Cisplatin) alone costing upwards of £51,000 for a short 4 week course^[4].

Altogether, SCLC makes up almost 25% of lung cancers (just under 325,000 new cases a year, out of 1.3 million total new cases of lung cancer worldwide^[5]), which are the most malignant cancers in males^[2]. Taking £51,000 as the average cost of treatment, with ~50% of patients at a stage suitable for chemotherapy, this leads to a staggeringly huge cost of £8,450,000,000 (eight billion, four hundred and fifty million pounds) per year worldwide; almost 10% of the UK NHS budget, without taking into account subsequent treatments for cancers and other disorders caused as a side effect of cisplatin.

Currently, there is great interest in the use of nanotechnology in medical diagnosis and treatments. Such technologies include microscopic scaffolds to form a framework for tissue growth and manipulation, DNA scanning diagnostic units and, possibly most usefully, the use of Nanoparticles (most commonly buckminsterfullerene) to operate as a drug delivery system within the body. Buckminsterfullerene can be used to

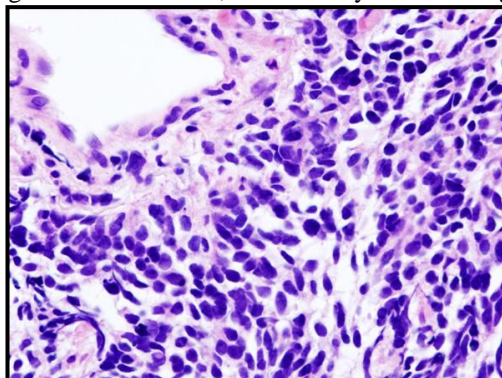


Figure 1: Microscopic view of a Core Biopsy sample of lung tissue showing presence of SCLC. Note enlarged Nuclei, indicating virulent reproduction

deliver any drug to a cell; the buckminsterfullerene itself will be broken down and the drugs it contains will be released. Here, we will explore the possibility of the delivery of Chemotherapy drugs to cells.

Discussion

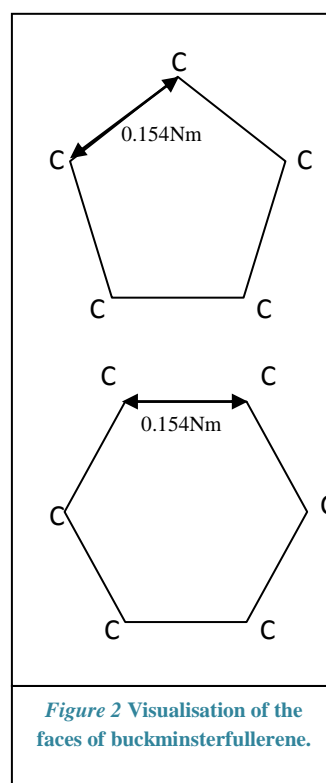
One of the characteristics of SCLC tumours is the presence of what is known as the cluster w4 antigen^[3]. This antigen is not only present on SCLC tumours but all Small Cell cancers, and is not found elsewhere in the body. The w4 antigen has an equivalent antibody, SWA11, which will lock onto Cluster w4 and has a strong toxic effect, causing rapid cell death. Normally, this should allow the immune system to mount a defence against the cancers, but the time taken for a guaranteed immune response may be enough to allow the cancer to progress to the incurable extensive stage, and patients who have undergone recent transplant surgery and are taking immunosuppressant drugs, or sufferers of diseases such as AIDS will be unable to mount an effective defence.

The technique of creating monoclonal antibodies through genetically modified rodents is receiving a large amount of scientific attention. It allows for the bulk production of huge numbers of specifically tailored antibodies in a very short time. Two methods can be used to create monoclonal antibodies: a modified B-lymphocyte can be created using a viral vector (such as the Epstein-Barr virus) to introduce relevant carcinogenic (for effective cell immortality) and antibody coding sections. The result is a cell which will last almost indefinitely and produce a constant stream of antibodies which can be separated for use by liquid-liquid extraction or by centrifugation^[6].

The other method involves injecting cells from the cancer patient into a mouse and waiting for corresponding B cells to be produced. Then, a cancerous B-lymphocyte (specially selected to be sensitive to the HAT cell culture medium) and a healthy B-lymphocyte are taken from the mouse. An equal mix of the cells can be mixed with polyethylene glycol^[7], to temporarily break down the phospholipid bilayer, and allowed to reform to make Hybridomas. The resulting cells are placed on a HAT culture. Cancerous B-lymphocytes that have not undergone fusion will die due to their sensitivity to the medium, whilst ordinary B-lymphocytes will naturally die after a short period, leaving only antibody producing hybridomas. This process gives the same results as the other.

Although the latter method is simpler and cheaper than the former (involving no genetic modification), Hybridomas produced by the latter method will produce non-human antibodies which will provoke an antiglobulin response^[8]. This could not only cause severe problems for the patient, but also result in the effectiveness of the antibody treatment being decimated as large numbers of them are destroyed before they successfully act upon target cells. In this case, it is likely that either the antibody will simply be destroyed, which, catastrophically, will result in toxic buckminsterfullerene molecules filled with further toxic chemotherapy drugs being released into the blood stream without any kind of functional targeting mechanism. It is also possible, should the molecules come into contact with phagocytes, that the chemicals would be consumed, therefore killing the phagocyte but reducing the side effects to the patient.

By either of these methods, using small cell carcinoma cells from a patient (for example, from a core biopsy sample; as long as the relevant antigen is present, it can be used) large numbers of SWA11 antibodies can be produced. The Buckyballs can be formed in cisplatin and Paclitaxel so that the drugs are naturally encased, or the drugs can be inserted manually through a fullerene carbon nanotube. The latter method is possible but difficult due to the small size of the carbon-carbon bond: effectively, the molecule has to be 'stretched' in order to accommodate the entry of the chemotherapy drugs. The bond length of a C-C bond is 0.154 Nm ^[9], so the size of one gap in the side of a buckminsterfullerene molecule could be thought of as the area of a regular pentagon 0.154 Nm on a side, with a width of 0.237 Nm ^[10]. Using these values, we can find the area of one pentagonal 'hole' in a buckminsterfullerene molecule to be 0.0408 Nm^2 ^[10]. The size of another gap in the side of a buckminsterfullerene molecule could be thought of as the area of a regular hexagon



0.154Nm on a side, with a width of 0.308Nm (as the width of a hexagon is twice its side length). Using these values, we can find the area of one hexagonal 'hole' in a buckminsterfullerene molecule to be 0.062Nm^2 ^[12]. Cisplatin is a symmetrical molecule consisting of a central platinum atom bonded to an NH₃ group on one side (the platinum bonds directly to the Nitrogen) and to a Chlorine atom on the other. The Pt-Cl bond is 0.2324Nm in length, whilst the Pt-N bond is 0.1967 Nm. Combined, this makes a molecular width of 0.4291Nm, considerably larger than the size of both gaps. This means that a Cisplatin molecule in a Buckyball will be unable to escape until the molecule is broken down (as naturally occurs inside the cell).

Once the Cisplatin molecule is inserted, the Buckyball can be attached to a SWA11 antibody via a ligand; an intermediary ion which will attach to a metal or a carbon atom^[11]. The drug is now ready, and can be administered by injection (for very small doses) or by intravenous drip. The drug will then circulate in the bloodstream until it comes into contact with the tumour in question. Once this contact occurs, the surface antibody system will attach to the antigens on the cells, moving the Buckyball-cisplatin complex with it. Once at the site of the tumour, the buckminsterfullerene will be taken up into the cell by either passive diffusion or active uptake, and broken apart within the cytoplasm^[13]. Once the cisplatin has reached the desired area of the cell, it attaches to the DNA at several points and causes cross linking to occur^[14], the adducts of which distort the helical structure of the DNA. The distortion is such that the strand becomes "kinked"^[13]. This shape change activates the natural DNA repair mechanisms that normally occur during the rapid mitosis present in tumour cells. Once DNA repair has been found to be impossible, the mechanism will activate cell apoptosis, causing rapid and total atrophy of the tumour.

This delivery system will ensure smaller doses of chemotherapy drugs are required, as less of the drug is absorbed by healthy tissues or degraded in the bloodstream^[15]. The targeting ability of the antibody, combined with the protective and insulating properties of the buckminsterfullerene, will ensure that very little of the drug causes damage to systems not targeted. Thanks to this reduced effect on tissues, the side effects suffered by patients mentioned above will be dramatically reduced. The side effects are very well understood in the case of cisplatin: side effects increase in number and severity predictably and proportionately to an increased dosage^[16]. Because the dosage can be so dramatically reduced, therefore, we can say accurately how much less severe the side effects will be. Thanks to this predictability, assessing patients for chemotherapy will be made considerably easier. The reduced dosage will therefore mean that patients who previously couldn't be considered for chemotherapy thanks to underlying conditions or natural fragility will now be able to receive life saving treatment that may have weakened or killed them beforehand.

Finally, the system will allow for the treatment of undiagnosed secondary stage cancers that have metastasized to different locations within the body. Since the drug will not release itself to other tissues, active chemotherapy drugs will continue to circulate around the body until they come into contact with the antigen they are programmed to respond to. When this occurs, they will attack the tumour. This means that a dose of chemotherapy could entirely prevent secondary tumours from gaining a foothold, or reduce their size until diagnosis takes place.

Conclusion

Much research has already been done into increasingly dramatic and effective methods of cancer treatment. We are starting to probe at the limits of conventional medicine. However, the advent of nanotechnology presents us with a multitude of new avenues for exploration. The system we have described above will open the opportunity for chemotherapy use for hundreds of people who previously would have been denied treatment by necessity rather than choice.

The effects on the NHS could be profound. With the dosage of chemotherapy drugs reduced, and side effects dramatically reduced, we could even see outpatient clinics being able to administer chemotherapy in the same way that one may administer dialysis treatment, with patients being allowed to return to their homes between treatment rather than being confined to a hospital bed for reasons of safety. This would not only greatly increase patient morale (which can, of course, contribute to a faster healthy recovery) but also free up hospital beds for other conditions and funding for other treatments, as well as reducing the numbers of repeat patients admitted as

a direct result of chemotherapy treatment. In such a time of economic downturn, with the NHS budget being carefully scrutinised, any money saving strategy should be thoroughly embraced, especially when it carries with it so many other benefits.

Of course, the treatment is not perfect, and the method needs refining. Greater research into monoclonal antibodies is needed, for instance, to bring the price down. Preferably, we would reach the stage where a culture of hybridoma organisms can be constantly producing antibodies, rather than the current inefficient batch system. But with the ongoing efforts of charitable organisations, and the continued generosity of the government regarding medical research, we could see the funding required for such refinements easily supplied.

The administration cost is also not to be shirked at. This is almost entirely new as a treatment, and extensive medical trialling would be required before it could be introduced as a standard treatment. If it was successfully implemented, NICE would also be required to change the administrative and treatment protocols for cancer diagnosis, and some retraining of professionals *may* be required.

But, weighing the potential gains against the expense involved, we believe the treatment to be an entirely viable option for the future. We would recommend that the use of buckminsterfullerene in drug delivery receives all the attention and funding it so rightly deserves.

[¹] Article published on the American Cancer Society website: “*Hormone production by cultures of small-cell carcinoma of the lung*”, written by G. D. Sorenson MD, O. S. Pettengill PhD, T. Brinck-Johnsen PhD, C. C. Cate PhD, L. H. Maurer MD

[http://onlinelibrary.wiley.com/doi/10.1002/1097-0142\(19810315\)47:6%3C1289::AID-CNCR2820470610%3E3.0.CO;2-B/pdf](http://onlinelibrary.wiley.com/doi/10.1002/1097-0142(19810315)47:6%3C1289::AID-CNCR2820470610%3E3.0.CO;2-B/pdf)

[²] “*Lung Cancer, Oat Cell (Small Cell)*”, written by Irfan Maghfoor, MD

[³] “*Immunolocalisation and imaging of small cell cancer xenografts by the IgG2a monoclonal antibody SWA11*”, written by A. Smith, R. Waibel, G. Westera, A. Martin, A. T. Zimmerman, and R. A. Stahel.

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2246986/>

[⁴] University of Virginia

<http://faculty.virginia.edu/metals/cases/east3.html>

[⁵] Cancer Research UK research.

<http://info.cancerresearchuk.org/cancerstats/types/lung/incidence/>

[⁶] *A haptenspecific chimeric immunoglobulin E antibody which exhibits human physiological effector function.*

Neuberger MS, Williams GT, Mitchell EB, Jouhal SS, Flanagan, JG, Rabbitts TH (1985)

[⁷] *Comparison of polyethylene glycols as fusogens for producing lymphocyte-myeloma hybrids*

Richard D. Lane, Robert S. Crissmana and Mary F. Lachmana (1984)

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T2Y-476NCPJ-VV&_user=10&_coverDate=08%2F03%2F1984&_rdoc=1&_fmt=high&_orig=search&_origin=search&_sort=d&_docanchor=&_view=c&_searchStrId=1654988297&_rerunOrigin=google&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=a47a7d77de6fbad41fe4a353d820f178&searchtype=a

[⁸] *Tolerance to Rat Monoclonal Antibodies*

R J Benjamin, S P Cobbold, M R Clarke, H Waldmann

<http://jem.rupress.org/content/163/6/1539.abstract>

[⁹] *Bond Lengths and Energies*

University of Waterloo

<http://www.science.uwaterloo.ca/~cchieh/cact/c120/bondel.html>

^[10] Using values calculated by the Pentagon Dimensions calculator at <http://www.issi1.com/corwin/calculator/pentagon.html>

^[11] Common Ligands, http://en.wikipedia.org/wiki/Ligand#Common_ligands

N/B, source does not specify buckminsterfullerene, but does describe ligands as attaching to carbon atoms (the example given being in hydrocarbons).

^[12] Using values calculated by the Polygon Calculator at, <http://www.cleavebooks.co.uk/scol/calpolyg.htm>

^[13] Kennesaw State University
Modes of Action of Cisplatin, <http://www.chemcases.com/cisplat/cisplat12.htm>

^[14] MedTV
Cisplatin, <http://ovarian-cancer.emedtv.com/cisplatin/cisplatin.html>

^[15] Institute of Nanotechnology
Nanoparticles lessen side effects of potent cancer drug, <http://www.nano.org.uk/news/1169/>

^[16] Chemocare.com
<http://www.chemocare.com/bio/cisplatin.asp>