Therapeutic and Diagnostic Analysis of Cadmium Sulfide Quantum Dots Conjugated with Cysteine, Anticancer Drug and Folate

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ABSTRACT

Background: Nanotechnology is evolving in the field of cancer drug design and cancer therapy. The ultrafine nanoparticles that have a size range of 1-10nm are called Quantum Dots. The ability to fluorescence has deemed quantum dots as suitable candidate for diagnostic imaging. Upon proper structural arrangement these quantum dots can be engineered to detect specifically cancer cells and deliver anticancer agents to the targeted site for cancer cell apoptosis. Aim: Cadmium sulfide (CdS) nanoparticles conjugated with cysteine were prepared by wet method under high temperature and pressure. Methods: Folic acid receptor has shown overexpression in multiple types of cancers therefore the binding of folate to the composite ensures that only the cancer cells are targeted and normal cells remain unaffected. HeLa cells were opted to check the composite activity. Results: Composite showed 23% cell death in the first 2 hours of delivery. FTIR analysis confirmed conjugation of CdS with cysteine and further conjugation of dacarbazine and folic acid to cysteine. The molecules of folic acid attached to cysteine could be measured to be 2.545 x 10^15 molecules which were attached to 0.996 x 10^22 molecules of cysteine that were attached 1.66 x 10^20 molecules of CdS. Similarly, 2.3103 x 10^19 of doxorubicin were attached to 0.996 x 10^22 molecules of cysteine attached to 1.66 x 10^20 molecules of CdS. Furthermore, the cytotoxic nature of Cadmium Sulfide also gives an advantage two way cancer cell death. Conclusion: Quantum dots attached to specific ligands that bind to cancer antigens, antibodies, proteins, Receptors etc. can be used for effective diagnosis of cancer. They can act as probe and detect thousands of molecules simultaneously by using only a small quantity of nanoComposite.

Keywords: Cadmium sulfide, Cancer, Quantum dots, Dacarbazine

INTRODUCTION

Drug delivery through targeted methods to cancer cells in a new and promising field of cancer therapeutics that involves use of nanocomposite in combination with the drug to be delivered. The nanoparticles have shown promise in the field of targeted drug delivery and also have reduced the side effects caused by unselective delivery of the drug to healthy cells.

Fluorescent nanoparticles are of keen interest in cancer imaging and diagnosis (in vitro and in vivo), the fluorescent probes that are greater than 1nm size have the characteristics to be easily visualized in proper microscopic setup. One benefit of using these probes is that nanosized probes do not exceed the size of protein and therefore provide platform for high quality imaging at a molecular level. (Davis & Shin, 2008)

Quantum that are light activated give a choice to track the outcome of the treatment and ensure that the composite is bound to the target cells. (Iannazzo, et.al.,2021) Thus the immunohistochemical analysis will provide proof that the composite indeed has the ability to bind specifically to cancerous cells. (Sudimack & Lee, 2000)

The multifunctional nanomedicine is normally opted for the enhanced cancer treatment and it provides an integrated platform for various pathways of treatment. The multifunctional nanocomposite has the advantage of being size specific and targeting the cancer from various pathways. (Davis & Shin, 2008)

The increase in cancer fatalities is due to the heterogenous behavior of the cancer and the difference of phenotypic expression of cancer in different organs. this causes problems in cancer therapeutics. Furthermore, if the cancer has metastasized and formed metastatic colonies, this leads to further complications in the cancer treatment. (Sudimack & Lee, 2000) Paramagnetic and superparamagnetic agents e.g. iron oxide nanoparticles are currently used in cancer diagnosis by medical imaging. (Jun, Lee, & Cheon, 2008)

Nanocomposite for cancer therapeutics provides a new hope in the field of cancer research and treatment. But the health issues should also be considered. (Lu & Low, 2012)

Theranostic nanomedicine i.e. diagnostic and therapeutic functions are integrated into the same platform, have an advantage over conventional method as they provide simultaneous cancer treatment and diagnosis of the cancerous tissue or organ. These Nanocomposites have molecules for cancer diagnosis and treatment integrated into the same composite system. (Duncan & Spreafico, 1994)

CYS COOH NH Dacarbazine

Figure 1: Proposed model of nano Composite.
In this study, the nanoComposite of anticancer drug i.e. Dacarbazine was prepared conjugation to cadmium sulfide quantum dots coated with cysteine. The Cysteine gave the extra C and N terminal to which the anticancer drug and folic acid was conjugated. Attachment of folic acid was done to target folic acid receptors on cancer cells. HeLa cells were used to carry out a comparative study of composite activity with B-cells. The nanocomposite has shown enhanced activity against HeLa cells without harming normal cells.(Murrali, et.al, 2022)

**METHODOLOGY**

**Immobilization of cysteine on CdS quantum dots:** Modified wet method was used for preparation of cadmium sulfide quantum dots coated with cysteine at high heat and pressure with constant stirring spectrophotometric analysis of the particles was carried out to check the absorption pattern of the quantum dots. The final composite was termed Cd-Cys quantum dot.

**Conjugation Dacarbazine to Quantum dots:** Conjugation of Dacarbazine was carried out by using carbodiimide method. (M.S.Lodhi & Z.Q Samra, 2019). Particles were stored in PBS at 4 °C.

**Conjugation of folic acid with quantum dots:** Conjugation of folic acid with the quantum dots was carried out by Glutaraldehyde method (M.S.Lodhi & Z.Q Samra, 2019) and the sample was stored in PBS for further processing.

**Development of anti Dacarbazine antibodies:** Ovalbumin and BSA conjugated Dacarbazine was prepared by two step gluteraldehyde method. Anti Dacarbazine antibodies were prepared by subcutaneous immunizing the mice with BSA activated formulation of the anticancer drug on a weekly basis and then applying a biooster injection of the drug, Cardiac puncture was used to extract the blood from the mice from which serum was prepared and further analysed. The antibody presence was detected using immuno dot blot technique and ELISA. (Samra et al., 2012).

Mice were euthanized by chloroform and cardiac puncture was performed.

**Characterization of Composite attachment on HeLa cells:** Hela cell line was obtained from School of biological sciences, university of the Punjab, Lahore, Pakistan.

The activity of the nanocomposite was further analysed by MTT assay and Trypan blue test. Cell apoptosis assay was also performed using a control sample, a drug conjugated CdS sample and an only CdS without drug sample as comparative parameters to compare the activity of the Composite in controlled environment.

**Cell immunohistochemistry analysis** was also performed to confirm presence of drug on the Composite using Taxus red labeled secondary antibody.

**MTT assay:** MTT assay was carried out using standard protocol. Absorbance of samples was recorded at 570nm.

**Trypan Blue Test:** Cell density was determined using hemocytometer. Trypan blue assay standard protocol was used. Cell viability was found using formula:

\[
\text{Total no. of live cells in all squares / total no. of live cells} \times 100 = \text{cell viability.}
\]

**Cell apoptosis assay:** Cells were seeded in 24 well plates and incubated overnight. Composite, drug, CdS were added into different wells (100ul) and incubated for 30 min, 1 hour, 1h 30min and 2 hours and control was processed the same way. All the cells were stained by 0.4%trypan blue and cell viability of each sample was checked by the following formula:

\[
\text{Total no. of live cells in all squares / total no. of live cells X total no of dead cells X 100} = \text{cell viability.}
\]

**Cell immunohistochemistry:** Overnight seeding of the cells was carried out in a microwell plate. The seeded cells were then suspended in the cell culture media with the help of micropette. 10ul of cells were added onto albumin coated slide and 3 slides were made. 100ul of 1% gluteraldehyde was added onto the slides and slides were incubated in humidified chamber for 5 minutes (cell fixation). Fixed cells were washed with PBS. CdS final Composite (100ul) was added in all the slides and incubated for 1 hour at room temperature. Samples were washed with PBS and visualized under fluorescent microscope. Skim milk TBS for blocking was added in the 2nd and 3rd slide and incubation was carried out for 1 hour. Then primary antibody (mouse anti drug antibody) 100ul was added and incubated for one hour. Slides were washed with PBS. Then Taxus red conjugated secondary antibody onto both slides and incubated for 1 hour. Slides were washed and observed in fluorescent microscope.

**Immunohistochemistry:** Immunohistochemistry was performed on formalin fixed mouse epithelial tumor sample. Fixed tissue sample was visualized under microscope without any dye, with taxus red and with simple DAP staining to compare binding of Composite with normal tissue sample.

**Fixation and Embedding:** Formaldehyde fixed tumor tissue samples (tumor of treated mice, tumor of untreated mice, liver and kidney of both normal and tumor mice) were cut into cubical pieces of 4mm width. These tissue samples were then separately added in 5% sucrose in Tris buffer saline (TBS) and kept in shaking incubator 6 hours at room temperature. After 6 hours sucrose solution was discarded and same procedure was carried out with increasing quantity of sucrose i.e. with 10%, 20% and 30% sucrose solution in TBS. After dehydration of tumor samples the tissue sections were embedded in optimum cutting temperature compound (OCT) in the molds prepared from aluminum foil and molds were kept at -20 °C.

**Section cutting:** Sections of frozen tissues were cut by using cryostat at -10°C using OCT compound. Sections were transferred on albumin coated slides and stored at -20°C in slide in rack for further analysis.

**Immunohistochemistry:** Slides having tissue samples were processed for immunohistochemistry. Firstly blocking was carried out by adding a drop of 3% BSA on each tissue section and incubates for 1 hour. Then washing was carried out by TBS and slides were incubated with CdS nanoComposite and incubated for 1 hour at room temperature, the slides were visualized under fluorescent microscope.

**RESULTS**

**Spectrophotometric analysis of CdS particles conjugated with cysteine:** Spectrophotometric analysis of cadmium sulfide quantum dots coated with cysteine was carried out at different wavelengths to analyse the wavelength at which the sample will exhibit maximum absorption.

**Figure 1.1 Graphical analysis of quantum dot spectrophotometric test**

**Microscopic analysis of CdS:**

![Fig. 2.1: Microscopic analysis of CdS](image-url)
a. CdS crystals in white light
b. CdS nanoparticles in white light
c. CdS nanoparticles in infrared light

CdS nanoparticles were synthesized and characterized using various spectroscopic techniques. The nanoparticles were found to be biocompatible and could be used as carriers for anticancer drugs.

3. Stoichiometry analysis:

- **Cysteine**: 0.04g of CdS contain 1.66 × 10^20 molecules.
- **Folic Acid**: 2.545 × 10^16 molecules of folic acid were attached to 0.996 × 10^22 molecules of CdS.
- **Dacarbazine**: 2.3103 × 10^13 of dacarbazine were attached to 0.996 × 10^22 molecules of CdS.

**FTIR Analysis:**

Figure 3.4.1: FTIR analysis of CdS + Cysteine+Dacarbazine+Folic acid

Figure 3.4.4 Binding of folic acid and dacarbazine to the Composite is shown in the form of peaks in both the specific regions of the FTIR analysis graph thus showing positive formation of the Composite for further analysis.

**Synthesis of anti dacarbazine antibody in mice:** Immunodot blot of the mouse serum indicated that the antibody present in mouse serum is mouse anti dacarbazine antibody.

**ELISA to detect anti dacarbazine antibodies:** Positive result of ELISA using enzyme linked antibodies indicated presence of mouse anti Dacarbazine antibody in mouse serum.

**MTT Assay:** Color change was observed after addition of MTT reagent and detergent reagent in test and control sample. Intensity of color depicts amount of live cells present in culture.

Absorbance of control at 570nm = A570 = 1.694
Absorbance of test at 570nm = A570 = 1.649

Decrease in absorbance indicates decrease in NADP synthesis which means that cell proliferation is decreasing on addition of nanoComposite and cell death is being carried out by the composite whereas cell proliferation is normal in control sample.

**Trypan blue staining:**

**Control sample**

Number of cells per square = (9+9+7+13)/4 = 9 cells
Cells per ml in control = 90000

**Test sample**

Number of cells per square = 10+8+7+12/4 = 9.25 apx 9 cells per square

**Number of live cells =3+4+5+3/4=3.75 apx 4 cells**

**Number of dead cells = 9-4=5 cells**

% Viability in test sample = (4/9) x 100 = 44.4%

**Cell apoptosis assay:**

**Table 2.1**

<table>
<thead>
<tr>
<th>Sample</th>
<th>% viability 30min</th>
<th>% Viability 1h</th>
<th>% viability 1h 30min</th>
<th>% viability 2h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug</td>
<td>85.6%</td>
<td>84.8%</td>
<td>82.9%</td>
<td>82.6%</td>
</tr>
<tr>
<td>CdS+Folic acid</td>
<td>87.3%</td>
<td>86.3%</td>
<td>85%</td>
<td>82.6%</td>
</tr>
<tr>
<td>CdS + Folic acid + drug</td>
<td>85.6%</td>
<td>83.7%</td>
<td>81.8%</td>
<td>77.9%</td>
</tr>
</tbody>
</table>

According to cell apoptosis assay the Composite is more potent in causing cancer cell death than CdS or Drug acting alone on the cancer cells. Composite shows 23% cell death within 2 hours of incubation.

**DISCUSSION**

Remarkable advances are being made in the field of nanotechnology every day. Bread fields of life and industry are now utilizing nanotechnological advancements to obtain better and specific results and products. Construction of nanomaterials that are precise and accurate requires years of research and data comparisons to get good quality product. It may be possible that in future nanorobots may be injected into human bloodstream that can locate and cure any problem in human body without the problem of disease aggravation. These theoretical robots may seem futuristic; however, currently researchers are working on individual nanoparticles which have the potential to alter the way cancer is detected and treated. (Freitas, 1999)

The nanoparticles can be designed to diagnose and treat the cancer cells simultaneously. This is done so by encapsulation or coating of the anticancer drug and detection molecule onto the nanoparticles.
nanoparticels is such a way that the composite binds specifically to the cancer cells and does not impact on the normal healthy cells of the tumor. Once the particles are in the tumor, they slowly release medication, destroying the cancer over a period of hours or days. (Farokhzad & Langer, 2006)

One of the advantages of the use of nanoparticels is that these utilize a comparatively low dose of the chemotherapeutic medicine as compared to conventional chemotherapeutic methods. This results in specific drug delivery and rapid treatment of cancerous tissues which is normally prolonged in normal chemotherapy treatment. Further the side effects of chemotherapy treatment can be minimized by this approach. (Frieler, et.al, 2021)

Nanoparticles become entrapped in the tumor, evading the healthy tissue, due to improper formation of blood vessels in tumor. Another approach for drug delivery utilizes encapsulation; these capsules slowly release drugs over a specific time period. Ligand-targeted nanoparticels can also be used for cancer treatment, which carry medication on the outside of the particles, then bind with cancerous cells, and finally release the drugs once inside the cancer. (Haolin Chen, et.al, 2021)

Nanoparticles have the ability to make medical images brighter thus the treatment can be directed according to the imaging of the nanoparticels and their effect on the tumor. This type of screening can be done for days and can reduce the cost of the treatment and the treatment can be targeted according to the imaging results of the previous nano dose. (Jain & Stylianopoulos, 2010)

Mulder et al. (2006) said that one of the negative aspects of the use of quantum dots is that they have the ability to form clumps and aggregate at a specific site. This can be avoided by the progress of encapsulation of the quantum dots in a surfactant polymer. The charge on the polymer will cause the particles to repel each other and therefore no clumping will be observed.

Quantum dots have a great deal of scope in cancer diagnosis as well due to their fluorescent nature. These particels can specifically bind to the cancer cells by use of a cancer specific ligand and the fluorescence of the particles in the biopsy sample will indicate the presence of cancer cells. This method can also be used to check through imaging if the treatment is effecting the tumor or not. This makes them an excellent candidate for cancer biomarker synthesis.

Quantum dots are mostly being studied for toxic analysis and imaging purposes not by conjugation of specific ligands there is reported delivery of drugs into brain tumors by quantum dots that can cross brain blood barrier. Cadmium tellurium quantum dots are also conjugated with antibodies for antibody mediated cellular cytotoxicity in cancer cells. Serrone, et al., (2000)

CONCLUSION

Quantum dots provide a promising platform for both diagnosis and treatment of cancer. By specifically binding to cancer cells and giving green fluorescence in normal white light, cadmium sulfide quantum dots conjugated with cysteine, folic acid and dacarbazine can be used for treatment of various cancer types including cervical cancer, malignant melanoma, non Hodgkin’s lymphoma, sarcoma and islet cell carcinoma of pancreas. This method of treatment is highly economical as it reduces the cost of treatment involving various combinations of chemotherapeutic drugs and radiotherapy and has less side effects as compared to conventional cancer therapeutics methods.

REFERENCES