Multi-Walled Carbon Nanotube-Doxorubicin Supramolecular Complexes for Cancer Therapeutics

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ABSTRACT

The potential of carbon nanotubes as carriers and delivery vectors for anticancer drugs was investigated. Herein, we propose MWNT-doxorubicin supramolecular complexes that can be developed for cancer therapy. The formation of such complexes is evidenced by a sharp decrease in the intensity of the doxorubicin fluorescence spectrum and takes place via π-π interactions with the MWNT backbone. In addition, the structural characteristics of the CNT-doxorubicin complexes were examined by transmission electron microscopy and showed well dispersed pluronic wrapped MWNT but clustered MWNT-doxorubicin structures. Very interestingly the doxorubicin-MWNT complexes exhibit enhanced cytotoxic activity compared to both doxorubicin alone and doxorubicin-pluronic complexes.

Keywords: carbon nanotubes, doxorubicin, supramolecular complexes, cancer.

1 INTRODUCTION

The field of drug delivery is being revolutionised by the discovery of novel nanomaterials such as carbon nanotubes (CNT) capable of traversing the plasma membrane [1] that allow the cellular uptake of small molecules and macromolecules (e.g. nucleic acids and proteins [2,3]). CNT are composed of carbon atoms arranged into graphene sheets rolled-up into tubular structures. Although existing anticancer drugs are potent molecules, their efficacy is hindered by their side effects which mainly relate to their non-discrimination between healthy and cancerous cells. For this reason the development of efficient delivery systems such as CNT with the ability to reach target sites to deliver existing potent drugs is a perquisite. The aim of this work was to investigate the formation of non-covalent carbon nanotube-doxorubicin supramolecular complexes and to assess its cytotoxic activity [4].

2 METHODS

2.1 Preparation of MWNT-Doxorubicin complexes

Pristine MWNT were dispersed using 1 % of tri-block copolymer (Pluronic F127) by bath sonication for 30 minutes into a final MWNT concentration of 1 mg/ml [5]. Pluronic MWNT -Doxorubicin complexes were prepared by mixing equal volumes of doxorubicin hydrochloride (20 μg/ml) with increasing MWNT concentration (10, 20, 40 μg/ml). The formed complexes had from $0.5x10^{18}$ to $2x10^{18}$ molecules of doxorubicin per mg of MWNT.

2.2 Fluorescence Spectrophotometry

Doxorubicin is a fluorescent molecule with a chromophore composing of three planar and aromatic anthraquinonic rings. Hence, the supramolecular interaction between doxorubicin and MWNT was studied by monitoring doxorubicin fluorescence using fluorescence spectrophotometery (Perkin Elmer Luminescence Spectrometer LS 50B).

2.3 Transmission Electron Microscopy

The structural characteristics of the doxorubicin – MWNT complexes were studied using transmission electron microscopy (TEM). The complexes were prepared as described above but by keeping the final MWNT concentration constant at 0.5 mg/ml in order to visualize the complexes while keeping the same number of doxorubicin molecules per mg of MWNT as used for the fluorescence analysis.

2.4 *In Vitro* Cytotoxicity Assay

The epithelial breast cancer derived MCF-7 cells were seeded in flat bottomed 96-well plates and were allowed to attach and grow by incubating them at 37º C in 5 % CO_2 for 24 hours. Cells were treated with pluronic MWNT-doxorubicin complexes at doxorubicin:MWNT mass ratio of 1:2, equivalent doxorubicin alone (600 nM), (pluronic 6.5 ng/ml) and CNT (615.5 ng) and the equivalent pluronic-doxorubicin. Control wells were treated with complete media. After 24 hours, the medium was removed and replaced by 120 μl of MTT/media (20 μl MTT + 100 μl complete media) and incubated for $3^1/_2$ hours to allow MTT reduction. A 100 μl of DMSO was then added to each well and left for 10 minutes at 37 ºC to allow complete solubilisation of the formazan product. The plate well was then measured for the optical densities at 570 nm using ELISA plate reader to determine the cell viability. This was represented as the percentage cell viability which is equal to (the optical density/ mean control)*100.

3 RESULTS & DISCUSSION

Figure 1 shows the quenching of doxorubicin fluorescence intensity as the final concentration of MWNT was increased from 5 to 20 µg/ml. Optimum interaction between doxorubicin and MWNT as evidenced by maximum quenching occurred at $0.5x10^{18}$ doxorubicin molecules per mg of MWNT. Interestingly, when the same number of doxorubicin molecules was mixed with Pluronic F127 alone, there was no decrease in fluorescence intensity as compared to that of doxorubicin in water, suggesting the non-interaction between the copolymer and the drug. The complex formation between the drug and MWNT can be explained by static quenching of doxorubicin molecules due to π -π stacking of doxorubicin aromatic chromophore and the carbon nanotube backbone. Interestingly, the affinity of the doxorubicin for self and hetero-association with various compounds with planar aromatic rings systems has been described before [6,7].

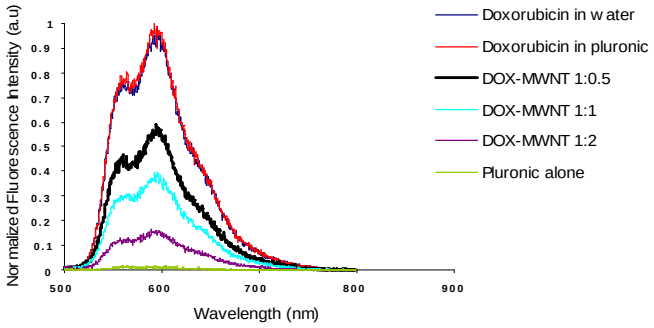

Figure 1: Normalised Fluorescence Intensity of MWNT-doxorubicin complexes. Final doxorubicin concentration was fixed to 10 µg/ml while increasing final MWNT concentration to 5, 10, 20 µg/ml. This is equivalent to the following mass ratios 1:0.5, 1:1 and 1:2.

Interestingly, TEM images show the strong interaction between doxorubicin and MWNT by the formation of supramolecular clusters (Figure 2). These are clearly observed as the number of doxorubicin molecules was decreased to $0.5x10^{18}$ as compared to the well dispersed and individualised pluronic wrapped nanotubes (Figure 2, MWNT alone).

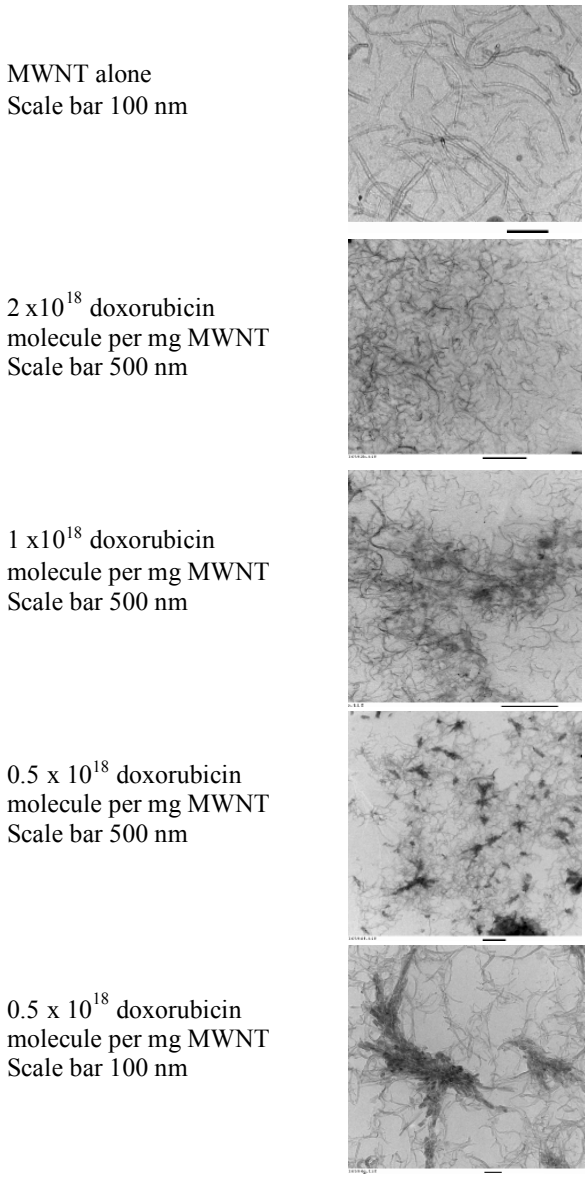

Figure 2: TEM images of doxorubicin MWNT complexes

Moreover, there was a statistically significant enhancement in the cytotoxicity of doxorubicin–MWNT complex as compared to doxorubicin alone and the Pluronic –doxorubicin with a p value of 0.016 and 0.0001 respectively (Figure 3). The enhanced cytotoxicity of doxorubicin when complexed with MWNT suggests that the nanotubes are mediating a more effective delivery of the drug molecules and improving its cellular uptake. Interestingly, no significant difference was observed between the Pluronic-doxorubicin and doxorubicin treated cells (p= 0.4931) suggesting the non-contribution of the

polymer in the enhanced drug cytotoxicity which correlates well with the fluorescence data suggesting no complexation between the Pluronic and doxorubicin.

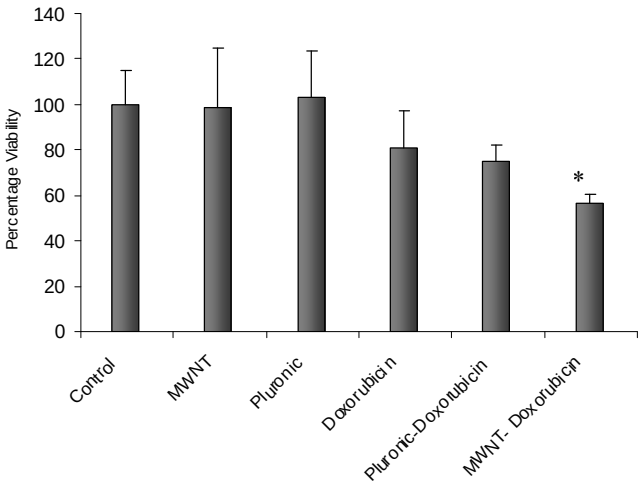

Figure 3: Percentage cell viability of MCF-7 cells after 24 hrs incubation. Doxorubicin-MWNT complex is at 1:2 mass ratio. Statistical significance between MWNT-doxorubicin and doxorubicin alone ($p < 0.05$) and Pluronic-doxorubicin ($p < 0.005$) respectively.

4 CONCLUSIONS

We have obtained strong evidence that supramolecular complexes can be formed between the anticancer drug doxorubicin and the copolymer coated MWNT as demonstrated by the sharp decrease in the fluorescence intensity of doxorubicin which is thought to be due to π -π interaction with the CNT backbone. Structural characteristics of the complexes were revealed by TEM. We have also shown that the doxorubicin-MWNT complex exhibit enhanced cytotoxic activity compared to doxorubicin alone and doxorubicin-Pluronic complexes. The mechanism for this enhanced activity is hypothesized to be due to the greater penetrative capacity of carbon nanotubes to cross the plasma membrane, however the exact mechanism of action is not yet fully elucidated.

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