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Research paper

Reversible Targeting and controlled release delivery of daunorubicin to cancer cells by aptamer-wrapped carbon nanotubes

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ABSTRACT

Aim: Single-walled carbon nanotubes (SWNTs) have been already used as drug carriers. In this study, we introduced sgc8c aptamer (this aptamer targets leukemia biomarker protein tyrosine kinase-7) to complex between Dau (daunorubicin) and SWNT to enhance targeted delivery of Dau to acute lymphoblastic leukemia T-cells (Molt-4).

Material and methods: Dau-aptamer-SWNTs tertiary complex formation was analyzed by visible spectroscopy and spectrofluorophotometric analysis. Dau release profiles from the complex were investigated in pH 7.4 and 5.5. For cytotoxic studies (MTT assay), Molt-4 (target) and U266 (B lymphocyte human myeloma, non-target) cells were treated with Dau, Dau-aptamer-SWNTs tertiary complex. Internalization was analyzed by flow cytometry. Targeted delivery of Dau was antagonized using antisense of aptamer.

Results : Dau was efficiently loaded onto SWNTs (efficiency ~157%). Dau was released from Dau-aptamer-SWNTs tertiary complex in a pH-dependent manner (higher release rate at pH 5.5). Flow cytometric analysis showed that the tertiary complex was internalized effectively to Molt-4 cells, but not to U266 cells. Cytotoxicity of Dau-aptamer-SWNTs tertiary complex also confirmed internalization data. Dau-aptamer-SWNTs tertiary complex was less cytotoxic in U266 cells when compared to Dau alone. No significant change in viability between Dau- and complex-treated Molt-4 cells was observed. Cytotoxicity of Dau-aptamer-SWNTs complex was efficiently and quickly reversed using antisense in Molt-4 cells.

Conclusion: Dau-aptamer-SWNTs complex is able to selectively target Molt-4 cells. The other advantages of this system are reversibility and pH-dependant release of Dau from its complex.

1. Introduction

Leukemia is an uncontrolled proliferation of blood cells and bone marrow, leading to death in many cases [1]. Acute lymphoblastic leukemia (ALL) is ranked among the most diagnosed malignancy in children [2].

Daunorubicin (Dau) is classified as anthracycline antitumor antibiotics. Dau is one of the most widely used chemotherapy agents in treatment of leukemia [3–5]. Clinical administration of Dau is limited by cumulative cardiotoxicity and myelosuppression, aside from its conventional side effects such as nausea, vomiting, and alopecia [4,5].

Tumor-specific delivery of anticancer drugs maximizes the efficacy of drugs and minimizes their off-target effects [6]. The aim of targeted delivery is to selectively accumulate drug in the target site [7,8].

In recent years, aptamers have been used as molecular targeting agents for specific drug delivery [9–11]. Originally discovered in 1990 [12], aptamers are single-stranded DNA or RNA nanomaterials. Their size could vary from 20 to 80 nucleotides [13,14]. Because of their three-dimensional structures, aptamers could selectively bind to a variety of targets ranging from small molecules to proteins and whole cells [12,15,16]. Aptamers are generated in an *in vitro* process called SELEX (Systematic Evolution of Ligands by Exponential Enrichment) [12]. Cell-SELEX has been recently developed to isolate cell-specific aptamers. So far, several aptamers have been generated by cell-SELEX for cancer cells such as liver cancer and small and non-small lung cancers [17]. No toxicity and immunogenicity has been reported for aptamers to date of preparation of this article. Beside selectivity and high affinity, aptamers are stable in heat and reduced condition. Moreover, aptamers can be chemically synthesized in large scale. Aptamers have

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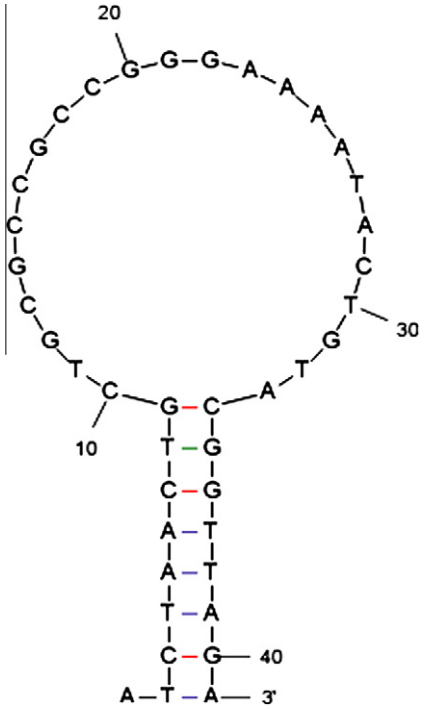

Fig. 1. Secondary structure of sgc8c aptamer predicted by mfold ($\Delta G = -2.24$) [20,21]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

potential applications in medicine such as therapeutic, diagnostic, or biosensor [15,16,18,19]. Sgc8c aptamer (Fig. 1) targets leukemia biomarker protein tyrosine kinase-7 (PTK7) [10,22,23]. Sgc8c aptamer can recognize target leukemia cells (T-cell ALL) with high affinity (k_d = 1 nM) among normal human bone marrow aspirates [10].

Discovered by Iijima in 1991, carbon nanotubes (CNTs) are molecular-scale tubes of graphitic carbon [24–26]. CNTs are classified structurally as single-walled carbon nanotubes (SWNTs) and multi-walled carbon nanotubes (MWNTs) [24,25]. Because of their unique physical and chemical properties, SWNTs have been the focus of interest in electronics, chemistry, biosensing, and drug delivery research projects [26–29].

In this study, we investigated the possibility of targeted delivery of Dau to Molt-4 cells (T-cell line, ALL) using SWNTs and sgc8 aptamer.

2. Materials and experiments

2.1. Cell culture

Molt-4 (C149, T-cell line, human ALL) and U266 (C151, B lymphocyte, human myeloma) were purchased from Pasteur Institute of Iran and cultured in RPMI 1640 (Euroclone) supplemented with 10% fetal bovine serum (FBS, heat inactivated, Gibco) and 100 units/ml penicillin–streptomycin (Sigma). All experiments were performed in FBS-free media.

2.2. Functionalization of SWNTs with aptamer

Sgc8c aptamer, 5′-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-3′, was synthesized using a Polygene DNA synthesizer (Polygene, Germany). Synthesized oligonucleotides were purified on polyacrylamide denaturating gel (7 M urea). Then, 350 mg/l SWNTs (Sigma, Cat. No. 704113) was mixed with 40 μM sgc8c aptamer in Tris–HCl buffer [tris(hydroxymethyl)aminomethane] (pH 7.4). Solution was sonicated for 2 h, followed by centrifugation for 2 h at 21,000*g*. The pellet comprising of impurities, aggregates, and bundle of nanotubes at the bottom of the centrifuge tube was discarded, and the supernatant was collected. Supernatant was centrifuged again. The concentration of the solubilized SWNTs (aptamer-SWNTs complex) was determined by its absorbance at 808 nm using a CECIL9000 spectrophotometer (Cecil company, UK) [30]. The morphology and size of aptamer-SWNTs was investigated by transmission electron microscopy (TEM) (LEO 912 AB, Germany).

2.3. Dau loading onto the nanotubes

A final concentration of 0.5 mM Dau was incubated at 37 °C for 16 h with 40 mg/l functionalized SWNTs in phosphate buffer saline (PBS) pH 7.4. Dau-aptamer-SWNTs tertiary complexes were collected by repeated ultracentrifugation at 130,000*g* until the supernatant became colour free. The amount of unbound Dau was determined by measuring absorbance at 488 nm, relative to a calibration curve recorded under the same conditions, allowing the drug loading efficiency to be estimated.

Moreover, to evaluate Dau loading, fluorescence spectra of solution of free Dau and Dau after incubation with aptamer-SWNTs complex (4 μM total Dau) were obtained using a Shimadzu spectrofluorophotometer (Shimadzu, Japan) [λ_{Ex} = 488 nm, λ_{Em} = 500–700 nm, Slit = 5 mm].

2.4. Dau release from the nanotubes

Dau-aptamer-SWNTs complex was incubated at 37 °C in either pH 7.4 or pH 5.5 PBS buffered solution. After 1, 2, and 3 days, the nanotube complexes were separated from the buffer by ultracentrifugation at 130,000*g*, and the concentration of released Dau in the supernatant was calculated by UV–Vis spectroscopy.

2.5. Flow cytometry analysis

Internalization of Dau and Dau-aptamer-SWNTs tertiary complex were measured according to Taghdisi et al. [11]. Briefly, Molt-4 and U266 cells were seeded in 12-well plates (2×10^5 cells per well). Cells were incubated in the presence of 2 μM (final concentration) of Dau and Dau-aptamer-SWNTs tertiary complex for 3 h. After centrifugation at 4000*g* for 4 min, the supernatants were removed and fresh culture medium was added to each well followed by incubation at 37 °C for 24 h. Cells were centrifuged at 4000*g* for 4 min, followed by incubation with trypsin-ethyleneaminetetraacetic acid (EDTA) (GIBCO cat. No. 27250-018) for 3 min, resuspended in PBS for Dau fluorescence analysis on a Partec PAS flow cytometer (Partec GmbH, Germany). The data were analyzed using WinMDI 2.9 software (downloaded from http://en.bio-oft.net/other/WinMDI.html).

2.6. MTT assay

According to Taghdisi et al., Dau at concentration of 0.5 μM was applied for MTT assay [11]. To monitor cell viability, Molt-4 and U266 cells (1.5×10^5 cells per well) were seeded in 96-well plates. Cells were incubated for 3 h with Dau and Dau-aptamer-SWNTs tertiary complex with the same Dau concentration (0.5 μM). Aptamer-SWNTs complex was used as control. Cells were centrifuged at 4000*g* for 4 min. The supernatants were removed, and fresh culture medium was added to each well. Cells were incubated for at 37 °C 72 h. Then, 10 μl MTT solutions were added to each well and mixed. After 3 h of incubation, 100 μl DMSO

(dimethylsulfoxide) was added to each well and absorbance was measured with a microplate reader (BioTek, USA) at 545 nm.

2.7. Antagonization of aptamer with antisense

Antisense against sgc8c, 5′-TAG ATT GAC GAC GCG GCG GC-3′, was purchased from Microsynth (Switzerland). To improve resistance to nuclease degradation in the cell culture medium, all of the nucleotides were 2′-O-methyl-modified. Molt-4 cells (1.5×10^5 cells per well) were seeded in 96-well plates. Different amounts of antisense against aptamer ranging from 0 to 9 equiv relative to the sgc8c aptamer were added to wells. Wells were treated with 0.5 μM Dau-aptamer-SWNTs tertiary complex and incubated for 3 h. After centrifugation at 4000*g* for 4 min, supernatants were removed and fresh culture medium was added to each well. Cells were incubated for an additional 72 h. Viability of the cells was measured as above.

2.8. Time-dependent effect of the antisense on the viability of cancer cells

Molt-4 cells (1.5×10^5 cells per well) were seeded in 96-well plates. Three equivalents of inhibitory antisense dose were added at different time points, 0, 1, 2, 3, and 4 h after addition of Dau-aptamer-SWNTs tertiary complex to Molt-4 cells. After incubating for 3 h, MTT tests were performed to measure viability of cells.

2.9. Statistics

Statistical tests were performed using the Student's *t*-test. Data are means ± SD, $n = 3$ independent treatments.

3. Results

3.1. Water soluble aptamer-SWNTs complex characterization

The SWNTs concentration in solution after functionalization with ssDNA was calculated to be 40 mg/l (≈11.5% of the starting nanotube suspension). The functionalized SWNTs are well dispersed. Both SWNTs and aptamer-SWNTs were characterized using TEM. The overall diameter of functionalized nanotubes are about 6 nm (Fig. 2b), which is greater than the diameter of SWNTs (about 4 nm, Fig. 2a). This increase is due to the wrapping of SWNT by aptamers, 1–2 nm [31,32].

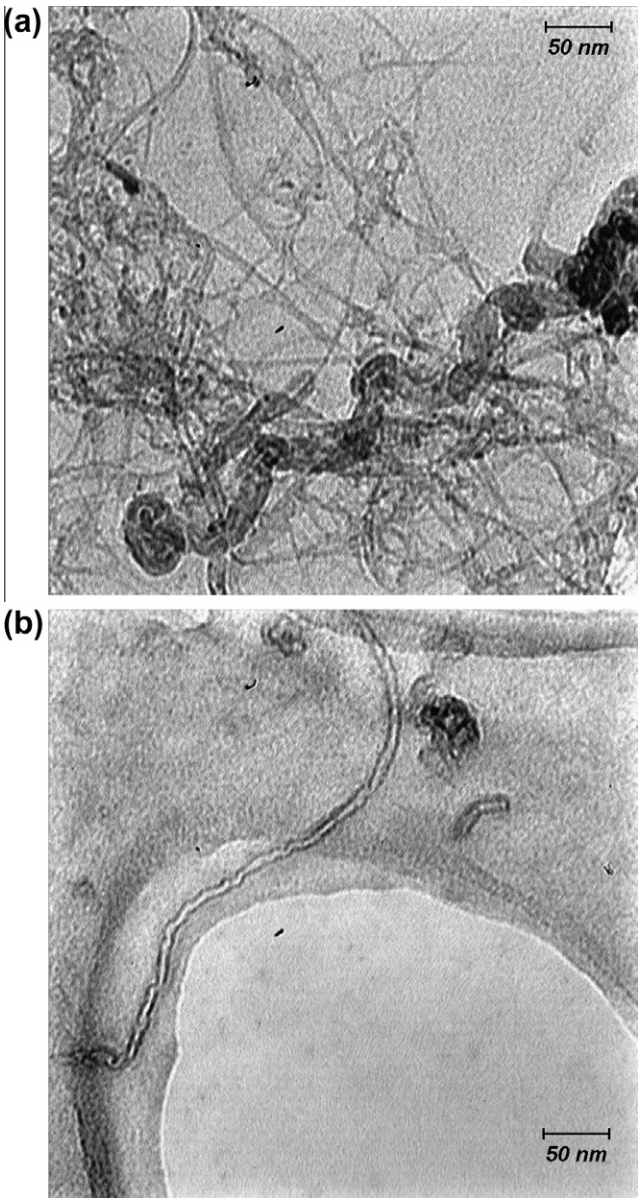

Fig. 2. TEM image of SWNTs (a) and aptamer-SWNTs (b).

3.2. Dau loading onto functionalized SWNTs

Dau-aptamer-SWNTs tertiary complex formation was monitored by visible spectroscopy (Fig. 3). Maximum absorbance of Dau in water occurred at 482 nm (λ_{max}). Upon the treatment of Dau with ssDNA-modified SWNTs, λ_{max} moved to 501 nm. This red-shift is usually interpreted as an indicator of interaction between Dau and SWNTS [33].

Dau-aptamer-SWNTs tertiary complex formation was also assessed by fluorometeric analysis. Dau has fluorescence feature, while SWNTs are known as strong quenchers [9,26,34]. Fig. 4 shows that Dau fluorescence spectra have been significantly quenched after incubation with aptamer-SWNTs complex (about 70%).

Drug loading efficiency onto functionalized SWNTs was calculated based on optical absorption. Dau loading efficiency (defined as the weight ratio of Dau to the functionalized SWNTs) onto nanotubes was found to be in excess of 157%.

3.3. pH-dependent Dau release from Dau-aptamer-SWNTs complex

Release of Dau-loaded nanotubes was pH-triggered. In slightly acidic solution of pH 5.5, corresponding to lysosomal pH, ~60% of Dau was released from complex in 72 h at 37 °C (Fig. 5), while Dau-aptamer-SWNTs tertiary complex was pretty stable after the same incubation at pH 7.4.

3.4. Internalization assay

The fluorescence FL2 histograms of Molt-4 and U266 cells after treatment with 2 μM Dau and 2 μM Dau-aptamer-SWNTs tertiary complex have been shown in Fig. 6. FL2 log intensity for Molt-4 cells after no treatment, treatment with Dau and complex was 18 ± 4, 50 ± 3 and 47 ± 4, respectively. FL2 log intensity for U266 cells after no treatment, treatment with Dau and tertiary complex was 20 ± 3, 53 ± 4 and 30 ± 4, respectively.

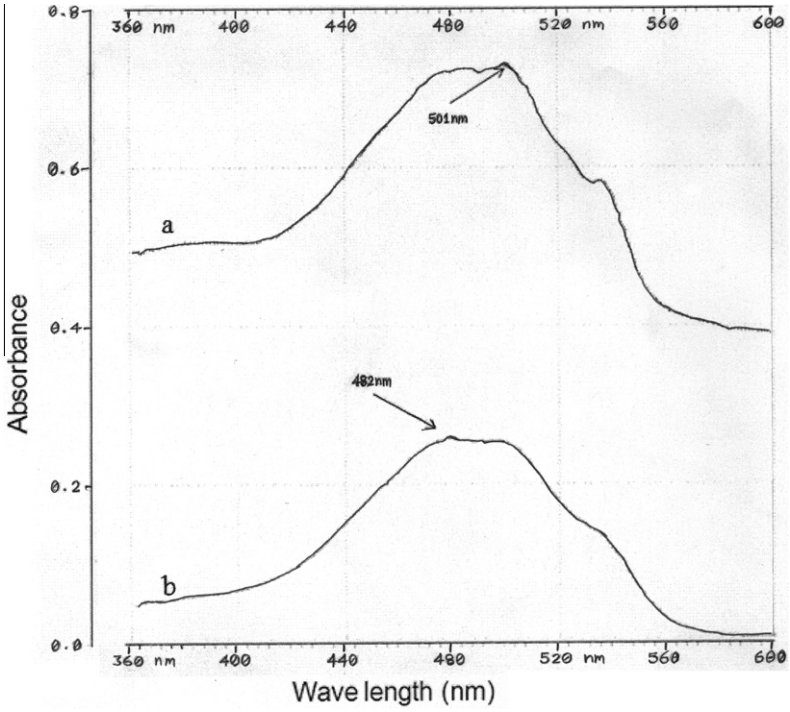

Fig. 3. Visible absorption spectra of Dau (b) and Dau-aptamer-SWNTs complex (a). Interaction between Dau with ssDNA-modified SWNTs moves Dau λ_{max} from 482 to 501 nm. The observed red-shift in spectra confirms Dau-aptamer-SWNTS complex formation.

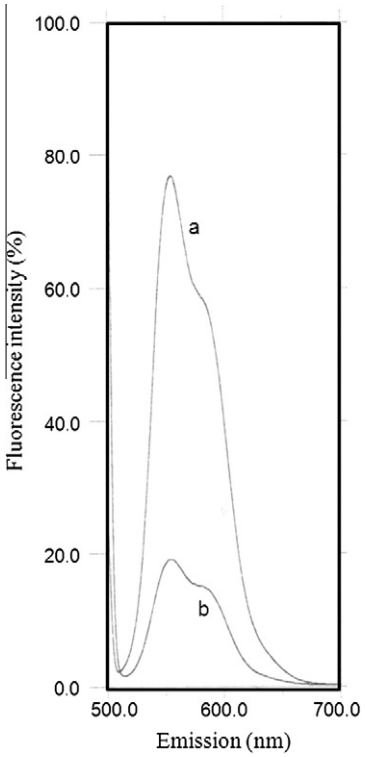

Fig. 4. Fluorescence spectra of Dau (a) and Dau after treatment with functionalized SWNTs (b). Dau fluorescence spectra (a) have been quenched (~70%) after incubation with aptamer-SWNTs (b).

3.5. Cell viability

MTT assay was performed to measure Molt-4 and U266 cells viability (Fig. 7). Molt-4 cell viability after treatment with aptamer-

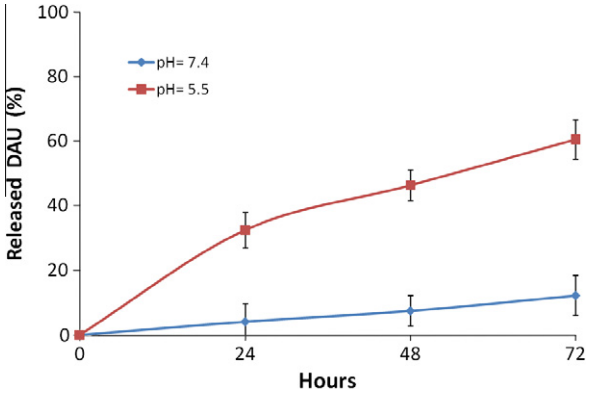

Fig. 5. Profile of Dau release from Dau-aptamer-SWNTs tertiary complex in PBS buffer at 37 °C at pH 7.4 (blue) and at pH 5.5 (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SWNTs, Dau, and Dau-aptamer-SWNTs tertiary complex were 93.9 ± 3.4%, 42.5 ± 3.2%, and 38.1 ± 5.4%, respectively. U266 cell viability after treatment with aptamer-SWNTs, Dau, and tertiary complex was 94.5 ± 4.6%, 41.3 ± 4.3%, and 78.9 ± 7.2%, respectively.

3.6. Effects of antisense on the viability of Molt-4 cells

Fig. 8 shows the antisense-dependent viability of Molt-4 cells treated with Dau-aptamer-SWNTs tertiary complex. Three equivalents dose of inhibitory antisense could retain the cell viability up to 90% after 72-h incubation with Dau-aptamer-SWNTs tertiary complex.

3.7. Time dependence of antisense effects on the viability of cancer cells

Co-treatment of Molt-4 Cells with antisense and Dau-aptamer-SWNTs tertiary complex showed that the highest viability (93.6% ±

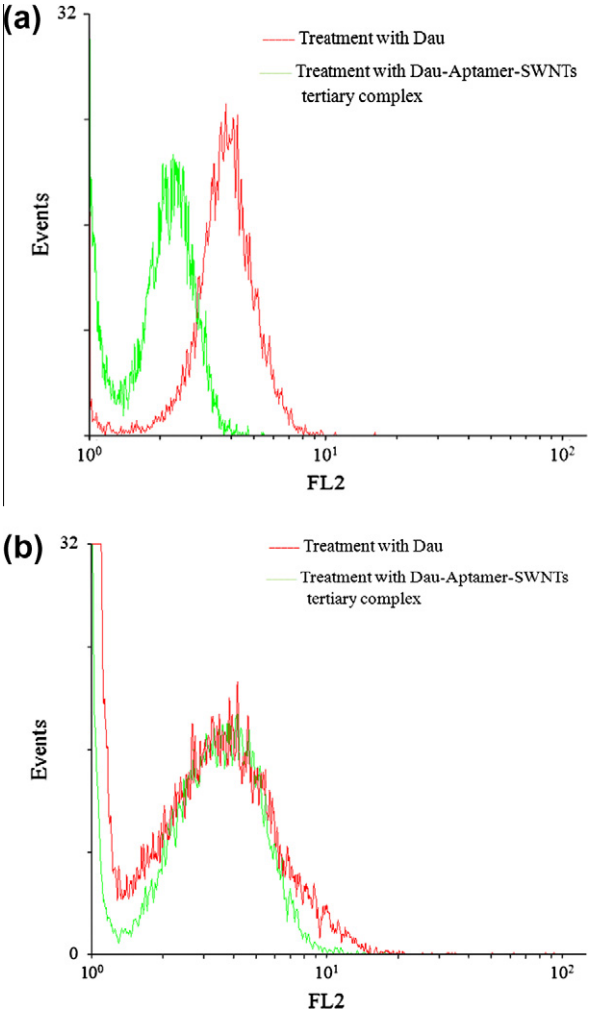

Fig. 6. (A) Flow cytometry histogram of U266 cells after treatment with Dau (red) and Dau-aptamer-SWNTs tertiary complex (green). (B) Flow cytometry histogram of Molt-4 cells after treatment with Dau (red) and Dau-aptamer-SWNTs tertiary complex (green).

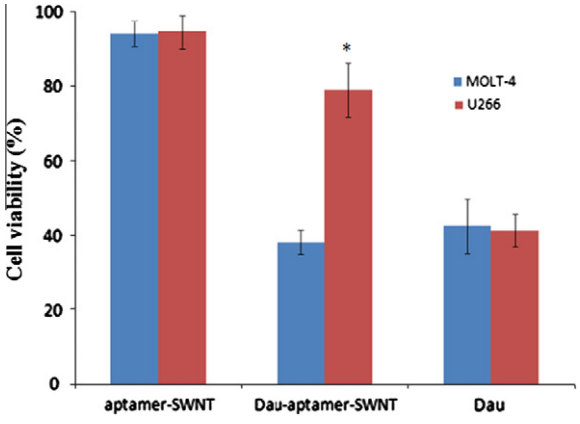

Fig. 7. Effects of Dau and Dau-aptamer-SWNTs tertiary complex on control and target cells viability (MTT assay). Cells were treated with aptamer-SWNTs, Dau, and Dau-aptamer-SWNTs tertiary complex for 3 h. After 72 h post-treatment, viability of the cells was assessed using MTT technique. * Indicates there is a significant difference between viability of Molt-4 and U266 cells after treatment with Dau-aptamer-SWNTs tertiary complex ($p < 0.005$).

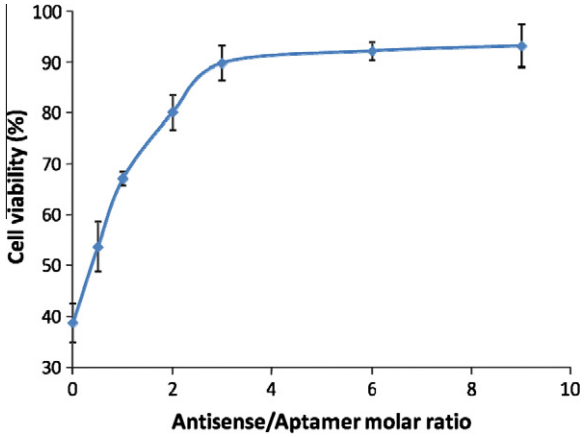

Fig. 8. Effects of antisense/aptamer ratio on viability of Molt-4 cells treated with Dau-aptamer-SWNTs tertiary complex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

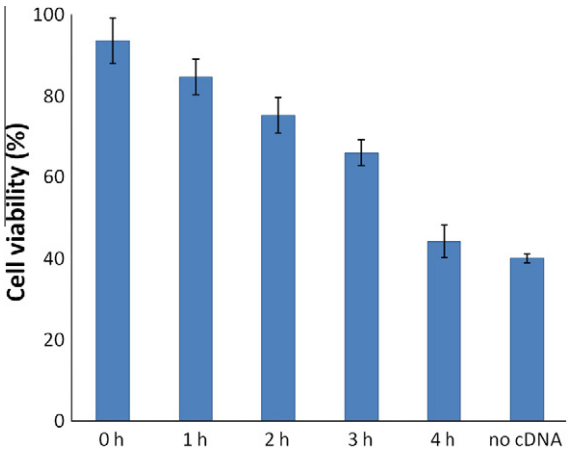

Fig. 9. Time-dependent effects of antisense on viability of Molt-4 cells treated with Dau-aptamer-SWNTs tertiary complex. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5.5) (Fig. 9). 1, 2, 3, and 4 h post-treatment of antisense to Molt-4 culture treated with Dau-aptamer-SWNTs complex, reduced the cell viability to 84.7 ± 4.3, 75.2 ± 4.4, 66.0 ± 3.1, and 44.2% ± 4.0, respectively.

4. Discussion

Surgery, chemotherapy, radiotherapy, and immunotherapy are common methods of cancer treatment. Chemotherapy is still the treatment of choice when a cancer is metastasized. Anticancer agents are associated with severe side effects because of their poor specificity. Targeted drug delivery can effectively minimize the adverse effect of chemotherapy and enhance their therapeutic efficacy by specific delivery of anticancer drugs to tumor area [35,36]. Targeted drug delivery system consists of a targeting ligand, a transport vehicle and the drug [37].

Bagalkot and his coworkers physically bound intercalating doxorubicin to PSMA aptamer for specific drug delivery to LNCaP cells ($PSMA^+$ cells). They used aptamer as both carrier and target agent to deliver doxorubicin to neoplasmic cells [9]. Using aptamers as cell-specific targeting ligand is much more economical in comparison with their application as therapeutic agent or vehicle because the quantity needed is much lower [13]. Therefore, in this

study, we used SWNTs as vehicle for drug loading and aptamer as a targeting ligand. SWNTs have characteristics, which make them suitable carriers for chemotherapy drug delivery. Large surface area of SWNTs makes them capable to adsorb drugs [38,39]. SWNTs do not intrinsically possess the recognition ability for selective targeting. This problem can be resolved by aptamer, as targeting agents [26]. Furthermore, SWNTs tend to form bundles that disperse poorly in aqueous solution [33]. Conjugation and functionalization with ssDNA like aptamers enhances SWNTs solubility and prevents them from aggregation [34]. SWNTs also protect aptamers from enzymatic digestion and elimination from the biological environment [26]. The synergy between SWNTs and aptamers may open a new window to targeted cancer therapy.

In this work, we used sgc8c aptamer as targeting ligand. Sgc8c aptamer identifies ALL T-cells with high affinity. Cell-specific internalization study showed sgc8c was internalized only in ALL T-cells [10]. SWNTs contain highly delocalized π electrons, so the surface of SWNTs can be easily functionalized thorough π–π interactions with compounds that possess a π-electron-rich structure [26]. Sgc8c aptamer non-covalently binds to SWNTs. π-stacking interactions between the nucleotide bases and the SWNT sidewalls are known to make a stable complex [26]. Dau also has a π-electron-rich structure, so that it can be adsorbed onto SWNTS with the same mechanism as aptamers.

Interaction between Dau and aptamer-SWNTs was monitored by measuring the quenching profile of Dau fluorescence after interaction with aptamer-SWNTs. More than 70% decrease in Dau fluorescence intensity confirmed formation of Dau-aptamer-SWNTs tertiary complex (Fig. 4). This tertiary complex was precipitated by ultracentrifugation and used for next experiment. Unbound Dau in supernatant was used to calculate the loading efficiency (∼157%).

While about 12% of Dau was released from Dau-aptamer-SWNTs tertiary complex in PBS (pH 7.4) at 37 °C after 72 h, switching to pH 5.5 speeded up the release of drug from its complex up to 60% under the same condition (Fig. 5). pH 5.5 resembles intracellular lysosomes, endosomes, or cancerous tissues condition. Lower pHs enhance hydrophilicity and, therefore, increase solubility of Dau by inducing protonation of $-NH_2$ groups. Less hydrophobicity reduces interaction between Dau and SWNTs [39].

Flow cytometry analysis showed that there is no preferential internalization of free Dau in Molt-4 and U266 cells ($p > 0.05$), because the mechanism of uptake for free drug is similar for both cell lines. However, treatment of Molt-4 cells with Dau-aptamer-SWNTs tertiary complex did not significantly change the intracellular concentration of free Dau in Molt-4 cells (target) in comparison with Dau alone ($p > 0.05$). While Dau-aptamer-SWNTs tertiary complex-treated U266 cells (non-target cell) showed a significantly lower fluorescence intensity in comparison with Dau-treated cells ($p < 0.005$) (Fig. 6), this is a strong indication that aptamer attached to complex can discriminate efficiently between target and non-target cells. Less entry into U266 cells may come from lack of aptamer binding site (PTK7, protein tyrosine kinase-7). Aptamer is a targeting agent and enters cells via receptor-mediated endocytosis (RME) [9]. Therefore, RME is invoked as the internalization mechanism for Dau-aptamer-SWNTs tertiary complex [27], while Dau alone unlike tertiary complex does not have a receptor-mediated uptake [9].

MTT assay also confirmed our internalization data (Fig. 7). Treatment of Molt-4 and U266 cells with aptamer-SWNTs did not have any significant effect on their cell viability ($p > 0.05$). Incubation of both cell lines with 0.5 μM Dau for 3 h significantly reduced their viability to about 42% ($p < 0.005$). Treatment of U266 cells with Dau-aptamer-SWNT complex significantly increased its viability to ∼78% ($p < 0.005$). The same treatment did not change the viability of Molt-4 cells ($p > 0.05$).

Aptamers function can be prohibited by their antisense (antidote), because the complementary base pairing disrupts the aptamer and target interaction. It has been demonstrated that antisense oligos are effective inhibitors for deactivating therapeutic aptamers in both animals and humans. These nucleic acid antidotes can be easily designed and synthesized. Moreover, their toxicity is predictable by BLASTing (Basic Local Alignment Search Tool, is a program that finds regions of local similarity between sequences). These advantages remove the safety issue associated with antidotes [13].

Study of the antisense-dependent cell viability showed the sgc8c antisense efficiently blocked Dau-aptamer-SWNTs tertiary complex uptake by Molt-4 cells and reduced the cells death. Our results showed that co-treatment of cell culture with Dua-aptamer-SWNTs complex and three equivalents dose of antisense could increase cell viability to ∼90% (Fig. 8).

Efficiency of antidotes therapy depends on how quickly they can show their inhibitory effects once they are applied. The result of time-dependent effect of the sgc8c antidote on the viability of Molt-4 cells showed that cells remained nearly intact when they were treated concomitantly with antisense and Dau-aptamer-SWNTs tertiary complex (Fig. 9).

5. Conclusion

Dau-aptamer-SWNTs tertiary complex delivery system is able to specifically deliver and internalize Dau to Molt-4 cells. Rate of Dau release in this system depends on pH of environment. In pH 5.5, rate of drug release increased about six folds. Moreover, application of antisense oligos against the aptamer can almost prevent delivery of Dau to Molt-4 cells. Therefore, this system can reduce cytotoxic effects of Dau by selective delivery and controllable release of this drug to tumor cells.

Conflict of interest

There is no conflict of interest about this article.

Acknowledgment

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References

[1] J.M. Kwan, A.M. Fialho, M. Kundu, J. Thomas, C.S. Hong, T.K. Das Gupta, A.M. Chakrabarty, Bacterial proteins as potential drugs in the treatment of leukemia, Leukemia Research 33 (2009) 1392–1399.
[2] L.K. Campbell, M. Scaduto, D. Van Slyke, F. Niarhos, J.A. Whitlock, B.E. Compas, Executive function, coping, and behavior in survivors of childhood acute lymphocytic leukemia, Journal of Pediatric Psychology 34 (2009) 317–327.
[3] L.F. Verdonck, H.M. Lokhorst, D.J. Roovers, H.G. Van Heugten, Multidrug-resistant acute leukemia cells are responsive to prolonged exposure of daunorubicin: implications for liposome-encapsulated daunorubicin, Leukemia Research 22 (1998) 249–256.
[4] G. Mojzisova, L. Mirossy, D. Kucerova, J. Kyselovic, A. Mirossay, J. Mojiz, Protective effect of selected flavonoids on in vitro daunorubicin-induced cardiotoxicity, Phytotherapy Research 20 (2006) 110–114.
[5] S. Duan, W.K. Bleibel, R.S. Huang, S.J. Shukla, X. Wu, J.A. Badner, M.E. Dolan, Mapping genes that contribute to daunorubicin-induced cytotoxicity, Cancer Research 67 (2007) 5425–5433.
[6] T. Betancourt, B. Brown, L. Brannon-Peppas, Doxorubicin-loaded PLGA nanoparticles by nanoprecipitation: preparation, characterization and in vitro evaluation, Nanomedicine 2 (2007) 219–232.
[7] K.K. Jain, Editorial: targeted drug delivery for cancer, Technology in Cancer Research and Treatment 4 (2005) 311–313.
[8] D.C. Li, X.K. Zhong, Z.P. Zeng, J.G. Jiang, L. Li, M.M. Zhao, X.Q. Yang, J. Chen, B.S. Zhang, Q.Z. Zhao, M.Y. Xie, H. Xiong, Z.Y. Deng, X.M. Zhang, S.Y. Xu, Y.X. Gao, Application of targeted drug delivery system in Chinese medicine, Journal of Controlled Release 138 (2009) 103–112.

[9] V. Bagalkot, O.C. Farokhzad, R. Langer, S. Jon, An aptamer-doxorubicin physical conjugate as a novel targeted drug-delivery platform, Angewandte Chemie – International Edition 45 (2006) 8149–8152.
[10] Y.F. Huang, D. Shangguan, H. Liu, J.A. Phillips, X. Zhang, Y. Chen, W. Tan, Molecular assembly of an aptamer-drug conjugate for targeted drug delivery to tumor cells, ChemBioChem 10 (2009) 862–868.
[11] S.M. Taghdisi, K. Abnous, F. Mosaffa, J. Behravan, Targeted delivery of daunorubicin to T-cell acute lymphoblastic leukemia by aptamer, Journal of Drug Targeting 18 (2010) 277–281.
[12] R. Stoltenburg, C. Reinemann, B. Strehlitz, SELEX-A (r)evolutionary method to generate high-affinity nucleic acid ligands, Biomolecular Engineering 24 (2007) 381–403.
[13] Z. Cao, R. Tong, A. Mishra, W. Xu, G.C.L. Wong, J. Cheng, Y. Lu, Reversible cell-specific drug delivery with aptamer-functionalized liposomes, Angewandte Chemie – International Edition 48 (2009) 6494–6498.
[14] E. Levy-Nissenbaum, A.F. Radovic-Moreno, A.Z. Wang, R. Langer, O.C. Farokhzad, Nanotechnology and aptamers: applications in drug delivery, Trends in Biotechnology 26 (2008) 442–449.
[15] K.A. Davis, Y. Lin, B. Abrams, S.D. Jayasena, Staining of cell surface human CD4 with 2′-F-pyrimidine-containing RNA aptamers for flow cytometry, Nucleic Acids Research 26 (1998) 3915–3924.
[16] L. Cerchia, F. Ducong, C. Pestourie, J. Boulay, Y. Aissouni, K. Gombert, B. Tavitian, V. De Franciscis, D. Libri, Neutralizing aptamers from whole-cell SELEX inhibit the RET receptor tyrosine kinase, PLoS Biology 3 (2005) 0697–0704.
[17] J.A. Phillips, D. Lopez-Colon, Z. Zhu, Y. Xu, W. Tan, Applications of aptamers in cancer cell biology, Analytica Chimica Acta 621 (2008) 101–108.
[18] N.S. Que-Gewirth, B.A. Sullenger, Gene therapy progress and prospects: RNA aptamers, Gene Therapy 14 (2007) 283–291.
[19] M. Blank, M. Blind, Aptamers as tools for target validation, Current Opinion in Chemical Biology 9 (2005) 336–342.
[20] J. SantaLucia Jr., A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics, Proceedings of the National Academy of Sciences of the United States of America 95 (1998) 1460–1465.
[21] M. Zuker, Mfold web server for nucleic acid folding and hybridization prediction, Nucleic Acids Research 31 (2003) 3406–3415.
[22] D. Shangguan, Y. Li, Z. Tang, Z.C. Cao, H.W. Chen, P. Mallikaratchy, K. Sefah, C.J. Yang, W. Tan, Aptamers evolved from live cells as effective molecular probes for cancer study, Proceedings of the National Academy of Sciences of the United States of America 103 (2006) 11838–11843.
[23] Z. Xiao, D. Shangguan, Z. Cao, X. Fang, W. Tan, Cell-specific internalization study of an aptamer from whole cell selection, Chemistry – A European Journal 14 (2008) 1769–1775.
[24] V. Sgobba, D.M. Guldi, Carbon nanotubes – electronic/electrochemical properties and application for nanoelectronics and photonics, Chemical Society Reviews 38 (2009) 165–184.
[25] M. Foldvari, M. Bagonluri, Carbon nanotubes as functional excipients for nanomedicines: II. Drug delivery and biocompatibility issues, Nanomedicine: Nanotechnology, Biology, and Medicine 4 (2008) 183–200.
[26] Z. Zhu, R. Yang, M. You, X. Zhang, Y. Wu, W. Tan, Single-walled carbon nanotube as an effective quencher, Analytical and Bioanalytical Chemistry (2009) 1–11.
[27] V. Raffa, G. Ciofani, O. Vittorio, C. Riggio, A. Cuschieri, Physicochemical properties affecting cellular uptake of carbon nanotubes, Nanomedicine 5 (2010) 89–97.
[28] H. Chen, C. Yu, C. Jiang, S. Zhang, B. Liu, J. Kong, A novel near-infrared protein assay based on the dissolution and aggregation of aptamer-wrapped single-walled carbon nanotubes, Chemical Communications (2009) 5006–5008.
[29] C. Tripisciano, K. Kraemer, A. Taylor, E. Borowiak-Palen, Single-wall carbon nanotubes based anticancer drug delivery system, Chemical Physics Letters 478 (2009) 200–205.
[30] N.W.S. Kam, M. O'Connell, J.A. Wisdom, H. Dai, Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction, Proceedings of the National Academy of Sciences of the United States of America 102 (2005) 11600–11605.
[31] D.M. Tasset, M.F. Kubik, W. Steiner, Oligonucleotide inhibitors of human thrombin that bind distinct epitopes, Journal of Molecular Biology 272 (1997) 688–698.
[32] H.M. So, K. Won, Y.H. Kim, B.K. Kim, B.H. Ryu, P.S. Na, H. Kim, J.O. Lee, Single-walled carbon nanotube biosensors using aptamers as molecular recognition elements, Journal of the American Chemical Society 127 (2005) 11906–11907.
[33] X. Zhang, L. Meng, Q. Lu, Z. Fei, P.J. Dyson, Targeted delivery and controlled release of doxorubicin to cancer cells using modified single wall carbon nanotubes, Biomaterials 30 (2009) 6041–6047.
[34] R. Yang, Z. Tang, J. Yan, H. Kang, Y. Kim, Z. Zhu, W. Tan, Noncovalent assembly of carbon nanotubes and single-stranded DNA: an effective sensing platform for probing biomolecular interactions, Analytical Chemistry 80 (2008) 7408–7413.
[35] J.K. Vasir, V. Labhasetwar, Targeted drug delivery in cancer therapy, Technology in Cancer Research and Treatment 4 (2005) 363–374.
[36] L. Zhu, Z. Huo, L. Wang, X. Tong, Y. Xiao, K. Ni, Targeted delivery of methotrexate to skeletal muscular tissue by thermosensitive magnetoliposomes, International Journal of Pharmaceutics 370 (2009) 136–143.
[37] X. Ye, D. Yang, Recent advances in biological strategies for targeted drug delivery, Cardiovascular and Hematological Disorders – Drug Targets 9 (2009) 206–221.
[38] X. Yu, Y. Zhang, C. Chen, Q. Yao, M. Li, Targeted drug delivery in pancreatic cancer, Biochimica et Biophysica Acta (BBA) – Reviews on Cancer 1805 (2009) 97–104.
[39] Z. Liu, X. Sun, N. Nakayama-Ratchford, H. Dai, Supramolecular chemistry on water-soluble carbon nanotubes for drug loading and delivery, ACS Nano 1 (2007) 50–56.