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Controlled delivery of dopamine hydrochloride using surface modified carbon dots for neuro diseases

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ABSTRACT

Delivery of therapeutic agents using water-soluble, highly biocompatible Carbon dots (C-dots) is an efficient strategy to control drug release under physiological milieu. Dopamine hydrochloride (DA), the most important inotropic vasopressor agent used in neurological diseases. In our study DA is anchored to water-soluble carbon dots for controlled release under mimicked in vitro physiological conditions. The tenure of the DA release at pH 7.4 was greatly extended to 60 h for C-dots–DA, in comparison with the control DA alone. The statistical calculation was used to comprehend the release pattern of the DA, which exhibited the pattern of Hixson–Crowell model of release. In order to understand the impact of the C-dots–DA conjugate under physiological conditions, Neuro 2A cells were taken under consideration. The conjugate C-dots–DA was found to be biocompatible against Neuro 2A cells. The survival rate was found to be 74% at maximum concentration of 9 $\mu g\,mL^{-1}$. In vivo toxicity was studied using thin section of tissues after staining with Hematoxyline and Eosin Yellow (H&E). As per microscopic observations, conjugates did not inflict any anatomical distortions or hostile effects on tissues. Body weight of mice was also taken into consideration after injecting 20 $\mu g\,mL^{-1}$ of nano-conjugates via tail vein. The impact of nano-conjugate on body weight was found to be negligible after 45 days of observation.

1. Introduction

Transporting the active pharmaceutical agents to the specific target requires smart strategies and safer delivery systems, in order to optimize the efficacy of the drug and its interaction with the targets. With the advancements in medical nanotechnologies especially the development of nanomaterials, the drug delivery profile in terms of absorption, distribution and elimination of the drugs from the human body can be improved by using the approaches of drug–nanomaterial conjugates [1,2]. Water-soluble nanomaterials are the most popular and effective drug delivery methods due to their high capability to circulate in the biological fluids and exhibit long sustained drug release during the course of treatment. Due to their excellent biocompatibility and facile synthesis protocols, C-dots can be widely used in biomedical applications, including drug delivery and cellular imaging [1]. However, it is extremely difficult to deliver and sustain the drug in human brains, due to the blood-brain barrier, which can highly restrict the drugs such as foreign peptides, chemicals, and proteins from getting in to the brain [2]. In recent years, to overcome this hurdle, C-dots attracted the attention from scientists due to their multifaceted applicability; like in biological imaging and labeling, photo-catalysis, as a matrix, in sensor design, etc. [3–8]. C-dots have also been applied in determining the critical micelle concentration of various surfactants [9] and as a matrix for detection of low molecular weight of compounds using matrix assisted laser desorption/ionization-mass spectrometry (MALDI-MS) [10].

In contrast to C-dots, semiconductor quantum dots such as CdS, CdSe, CdTe quantum dots are used for bioimaging, but impart toxicity due to Cd^{+} release [11,12]. The toxicological effects of quantum dots may be attributed to the free radical generation [13]. These heavy semiconductor metals are toxic to important

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human organs, such as liver and kidney because they can easily penetrate to these organs [14]. For a competent drug delivery system, intense functionalization strategies to attach neuro-drugs with carrier are required [15,16]. Due to the inherent surface decoration with —COOH, —OH and —NH_2 functional groups, a variety of chemotherapeutic agents can be anchored to the surface of C-dots. Additionally, the functionalized C-dots, under physiological conditions, are not only photochemically stable but also exhibit excellent physiochemical stability [17]. Hence, designing sophisticated nanoscale vehicles that can carry a bulk of drug to the specific drugs are desperately solicited.

In chemotherapy, the problem arises when the drug disperses before reaching to the target, leading to less permeation as well as off-target toxicity [18]. Major attention has been paid in controlling the shape and size of nanoparticles, so that they can carry drugs inside the fine blood capillaries [19,20]. Over the past few years, sustained release of neurotransmitters for dopamine has received major attention. The C-dots, due to their porous nature, have shown high drug loading capacities as well as excellent drug delivery properties [15,16]. The possibility of C-dots as a drug delivery vehicle to carry neuro-therapeutic agents is explored in the present study. This study is concerned with comprehending the in vitro release kinetics and attachment chemistry of the C-dots to DA. The release pattern of DA was found to follow Hixson–Crowell model, which are considered to be ideal for injectables.

In the present work, we have employed C-dots for effective drug delivery and analyzing its toxic effects, both in vitro and in vivo. Anchored DA to C-dots showed sustained release over 60 h than the bare DA (control). Both in vivo and in vitro toxicological studies have confirmed that the C-dots can be used as highly effective and biocompatible drug delivery systems.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (ACN), anhydrous citric acid and chloroform were purchased from J.T. Baker, USA. Dopamine hydrochloride and 3-(4,5 dimethyl-thiazol-2-ul-2,5-diphenly tetrazolium bromide were purchased from Sigma, USA. 96 wells and T-flasks were bought from Nune, Thermo Scientific, USA. Dulbecco's Eagle's medium was bought from Thermo Scientific, USA (DMEM, HyClone-containing 4 mM/L L-glutamine, 4500 mg/L glucose), fetal bovine serum (FBS) were purchased from Gibco, USA. Phosphate buffer saline (PBS), Trypsin-EDTA solution (17,000 U/L Trypsin mixed with 0.2 g L^{-1} EDTA) and pen-strep solution (10,000 U/L penicillin mixed with 10 mg/mL streptomycin) were bought from Lonza, Belgium. C-dots crystallinity was analyzed by XRD (Philip, The Netherlands). Whereas, Raman spectrometer with laser wavelength 760 nm was used. The water used for all experiments including the cleaning of glass wares were from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Instruments

Transmission Electron Microscope (JEOL, Japan) was used for the TEM images of C-dots. Carbon coated grids were drop casted using dilute solution of C-dots for the sample analysis. Surface passivation of C-dots was studied using Fourier transform infrared spectrometer (Perkin Elmer, USA), after drop coating C-dots solution on KBr pellets. The optical properties of C-dots were recorded by double beam UV–VIS spectrophotometer (Thermo, USA) between 200 and 550 nm. Same solution of C-dots was studied using fluorescence spectroscope (Hitachi, F-2700, Japan) at various excitation wavelengths (λ_{ext} = 200, 250, 300, 350 nm). The microtomy of mice organs were executed by a Hestion ERM 3000 semi-automatic Microtome (Australia).

2.3. Synthesis of C-dots

C-dots were synthesized in deionized (DI) water, in which 10 mg of citric acid was heated in a microwave for 2 min at 750 watt, then equal volume of 1 M NaOH and ethanol (3 mL) were added to the reaction mixture (1:1 ratio) and further microwave treatment followed by 1 min stirring to achieve a homogeneous solution. The resulting solution was transferred in a pre-activated dialysis bag (MW cut off 12–14 kD) of pore size 2–6 nm. To obtain nano-sized C-dots, they were dialyzed against nanopure water for 24 h; then, a yellow bright solution of pure C-dots was obtained which was used for all experiments.

2.4. Attachment of DA on C-dots (preparation of the nano-conjugate)

15 mg of cystamine hydrochloride was dissolved in 5 mL of DI water (1500 ppm). 40 μL from this stock solution was added to the 1 mL of pure C-dots and the total volume was adjusted to 3 mL with water. After stirring the reaction mixture for 3 h, 1 mL of DA solution (0.02 mg/mL) was added in the C-dots solution and further stirred at room temp for 3 h and maintain the concentration at 25 ppm.

2.5. Drug loading association efficiency

3 mL of the C-dots–DA conjugate was dialyzed against nanopure water. The conjugation efficiency of DA to C-dots was determined by the UV–vis spectroscopy, at 280 nm wavelength. The DA drug loading efficiency (DLE) was measured by the following equation.

$$\text{DLE} = \frac{\text{Theoretical amount of drug loaded} - \text{Free drug}}{\text{Theoretical amount of drug loaded}} \times 100$$

2.6. In vitro drug release studies

7 mL of either DA or C-dots–DA solutions were transferred in polycarbonate dialysis membrane (10 kD, Spectrum Labs, USA) add 90 mL Phosphate buffer solution (PBS) solution pH 7.4 was added. Samples were gently agitated in an incubator at 37 °C. To obtain a calibration curve for drug release profile, 3 mL of eluent was procured at the regular interval. Percent cumulative drug release (% CR) was determined by using the following formula:

$$\%\,CR = \frac{C_{0-f}}{C_o} \times 100 \quad (2)$$

where C_o and C_f are the final concentration of the DA.

2.7. Cytotoxicity study

In vitro cytotoxicity of C-dots, DA, and C-dots–DA conjugates were investigated by using MTT assay. MTT assay is based on the mitochondrial oxidoreductase enzyme, which converts pale yellow MTT dye to the violet crystals of formazan. Cells were seeded (5×10^6 cells mL^{-1}) in 96 microtiter plates (3596 cell culture microtiter plate corning star corp-Germany), incubated at 5% CO_2 at 37 °C for 24 h. C-dots, DA, C-dots–DA was added in the microtiter plate by replacing the medium and further incubating for 48 h. After which, these solutions were subjected to MTT dye (200 μg mL^{-1}) and incubated for 2.5 h at 28 °C to start the formation of formazan. The cell medium was again replaced with DMSO then vortexed slowly for a few minutes, in order to dissolve the formazan crystals.