Materials Science and Engineering C 65 (2016) 151–163

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Ceria-containing uncoated and coated hydroxyapatite-based galantamine nanocomposites for formidable treatment of Alzheimer's disease in ovariectomized albino-rat model

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ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 27 March 2016
Accepted 11 April 2016
Available online 14 April 2016

Keywords:
Nanoceria
Hydroxyapatite
Nanocomposite
Galantamine
Anti-Alzheimer agent

ABSTRACT

This paper upraises delivery and therapeutic actions of galantamine drug (GAL) against Alzheimer's disease (AD) in rat brain through attaching GAL to ceria-containing hydroxyapatite (GAL@Ce-HAp) as well ceria-containing carboxymethyl chitosan-coated hydroxyapatite (GAL@Ce-HAp/CMC) nanocomposites. Physicochemical features of such nanocomposites were analyzed by XRD, FT-IR, Raman spectroscopy, UV–vis spectrophotometer, N_2-BET, DLS, zeta-potential measurements, SEM, and HR-TEM. Limited interactions were observed in GAL@Ce-HAp with prevailed existence of dispersed negatively charged rod-like particles conjugated with ceria nanodots. On contrary, GAL@Ce-HAp/CMC was well-structured developing aggregates of uncharged tetragonal-shaped particles laden with accession of ceria quantum dots. Such nanocomposites were i.p. injected into ovariectomized AD albino-rats at galantamine dose of 2.5 mg/kg/day for one month, then brain tissues were collected for biochemical and histological tests. GAL@Ce-HAp adopted as a promising candidate for AD curativeness, whereas oxidative stress markers were successfully upregulated, degenerated neurons in hippocampal and cerebral tissues were wholly recovered and Aβ-plaques were vanished. Also, optimizable in-vitro release for GAL and nanoceria were displayed from GAL@Ce-HAp, while delayed in-vitro release for those species were developed from GAL@Ce-HAp/CMC. This proof of concept work allow futuristic omnipotency of rod-like hydroxyapatite particles for selective delivery of GAL and nanoceria to AD affected brain areas.

1. Introduction

Cerium oxide (CeO_2) nanoparticles, nanoceria, adopt a cubic fluorite crystal structure that has profound ability to reversibly bind oxygen and shifts between Ce^{4+} and Ce^{3+} states under oxidation and reduction conditions displaying plenty of oxygen vacancies in ceria crystal lattice, as shown in Eq. (1) [1,2].

$$CeO_2 \underset{\text{Reduction}}{\overset{\text{Oxidation}}{\rightleftarrows}} CeO_{2-y} + \frac{y}{2}O_2 \quad (1)$$

Such dual oxidation state of ceria has made it an ideal catalyst, and structural as well electronic promotors for various heterogeneous catalytic reactions in industrial, environmental and energy applications [3,4]. Nowadays, nanoceria is receiving much attention as a potential antioxidant agent that capable to scavenge the most prevalent reactive oxygen species (ROS) produced from various intracellular processes in living organisms such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (HO^-). Although the physiological levels of liberated ROS displayed beneficial role in signal transduction in nervous cells, the high reactivity of ROS and their ease accessibility from diverse environmental sources, like cigarette smoke, car exhaust and ultraviolet radiation from the sun, burst ROS levels in nerve cells weakening the ability of endogenous antioxidant systems, e.g. the non-enzymatic antioxidant like glutathione (GSH) and the enzymatic antioxidants like superoxide dismutase (SOD) and catalase (CAT), to neutralize ROS [5,6]. Such situation induced potential disturbance in the central nervous system metabolism resulting in a state of hazard oxidative stress [7]. Thus, nanoceria becomes attractive candidates for development in neurodegenerative diseases attributing to its high biocompatibility, possibility to participate in redox cycling reactions and capability to promote cell survival under conditions of oxidative stress, i.e. in vivo administration must be lower than 20 mg/kg animal body weight to avoid ceria accumulation in critical areas such as reticuloendothelial organs, and microcirculation or renal glomerular passage [2,8–10]. Furthermore, nanoceria was found to mimic the activity of both superoxide dismutase enzyme (SOD) in catalyzing the dismutation of O_2^- and catalase enzyme (CAT) in decomposing H_2O_2 to O_2 and H_2O [11,12]. More recently, it had been assumed by Heckman et al. that in vivo medicinal characteristics of ceria nanoparticles against free-radical mediated autoimmune neurodegenerative disease are

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http://dx.doi.org/10.1016/j.msec.2016.04.041
0928-4931/

hypothesized by customizing nanoceria of size ranging from 5 to 55 nm, often with relatively high negative zeta-potential (~−25 mV) [2]. Unfortunately, treatment of neurological disorders in brain with nanoceria is impeded by the incapability of those nanoparticles to cross the blood brain barrier thereby promoting oxidative stress in some settings [7].

One of the most common disease that arises from developed oxidative stress status is the Alzheimer's disease (AD), which is classified as highly complex and progressive neurodegenerative disordered disease in the elderly populations [13]. Such disease attacks cholinergic regions of the central nervous system causing cognitive dysfunction, severe behavioral disturbances and finally cell death [14]. Many factors are considered as the key pathological hallmarks of AD, such as the presence of plaques that originate from accelerated aggregations of Aβ-amyloid peptides in cerebrum and hippocampus tissues and the deteriorated activity levels of oxidative stress markers in brain tissues [6,15].

Galantamine (GAL) is an alkaloid initially isolated from the bulbs and flowers of *Galanthus caucasicus*, *Galanthus woronowii* and related genera [16]. It was first developed as an industrial drug that is used for treatment of myasthenia gravis, myopathy, residual poliomyelitis paralysis syndromes and decurarization [17]. Because of its ability to cross the blood brain barrier and to affect the central cholinergic function, GAL was run to treat AD [18]. It was found that galantamine prohibits the enzyme acetylcholinesterase from hydrolyzing the neurotransmitter acetylcholine, which plays a key role in improving memory and cognition, thereby alleviates the potential cause for existence of Aβ-amyloid peptides and AD [19,20]. Also, galantamine prevents mitochondrial dysfunction and endoplasmic reticulum stress [20,21]. Inappropriately, galantamine has been shown to suffer degradation under neutral, acidic, oxidative as well photolytic conditions yielding degradation products of very weak therapeutic efficiencies against AD [22].

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) is suggested to be one of the most promising drug delivery systems owing to its biocompatibility, bioactivity, elevated hydrophilic character, chemical stability against oxidative conditions and non-toxicity nature [23–25]. Further providence to the deliver action of HAp particles perhaps be conceivable by their coating with carboxymethyl chitosan (CMC), which exhibits prominent biocompatibility, biomedical activities and biodegradability [26,27].

The pressing need to discover a safe and therapeutic efficient drug against AD trigger us to manipulate a new class of anti-Alzheimer agent that comprised of three components, in which hydroxyapatite particles (either in uncoated or coated form) termed to upload nanoceria and galantamine species hoping to ameliorate the capability of nanoceria to cross blood brain barrier, and improve the stability of galantamine against the neutrality of brain intracellular pH as well the severe oxidative stress settings in AD infected tissues. This ternary nanocomposite has never been yet synthesized nor assessed in either medicinal or industrial fields.

The objectives of the concurrent article are summarized in recognition of the various physicochemical features of nanoceria-containing uncoated and coated hydroxyapatite-based galantamine nanocomposites, and evaluation of their therapeutic performances against AD induced in ovariectomized albino-rat model through studying the oxidative stress protein levels as well the histological characteristics of brain tissues.

2. Materials and methods

2.1. Reagents and animals

Anhydrous aluminum chloride ($AlCl_3$, as an inducer for Alzheimer's disease) was purchased from Fluka Chemicals (Ronkonkama, NY, USA) and galantamine hydrobromide (GAL, as traditional drug for Alzheimer's disease) was developed by Janssen Research Foundation under co-development and licensing agreement with the UK-based Shire Pharmaceuticals.

For preparation of ceria-containing uncoated and coated hydroxyapatite powders, calcium carbonate ($CaCO_3$), ammonium dihydrogen phosphate ($NH_4 \cdot H_2PO_4$), cerium sulphate ($Ce(SO_4)_2$), isopropanol and monochloroacetic acid were supplied by Sigma (St. Luis, MO, USA). Chitosan (degree of deacetylation: ca. 95%, molecular weight: ca. 80 kDa) was purchased from Heppe Medical. All other chemicals used in this research were of highest analytical grade. Deionized water was used throughout all the experiments.

Seventy adult female albino Wistar rats (6 months old) weighing 180–200 g were obtained from the Animal Breeding Colony of the Medical Research Centre, Ain Shams University, Cairo. All the rats were ovariectomized surgically in the Operation Room of Experimental Surgery Unit, Medical Research Centre, Ain Shams University as several lines of evidences suggest that changes in hormones after menopause play essential role in cognitive dysfunction and development of AD [28]. Ovariectomized rats were housed in five groups in ventilated metallic cages (58 cm × 38 cm × 20 cm, 14 rats per cage) on a layer of wood sawdust at constant room temperature of 55% humidity with natural day/night light cycle, food as well water ad libitum. All animals were allowed to acclimatize for 6 weeks post-surgical operation to adapt to laboratory conditions and to avoid any complications along the experimental courses. Animals cared for according to the guidelines of animal experiments by the Ethical Committee of National Research Centre (NRC), Egypt.

2.2. Preparation of nanoceria-containing uncoated hydroxyapatite powder

Nanoceria-containing calcium hydroxyapatite powder was prepared in a manner relevant to that operated for synthesis of conventional hydroxyapatite [25,29], following chemical equation:

$$10Ca(NO_3)_2 + 6(NH_4)H_2PO_4 + 14NH_4OH = Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 12H_2O. \quad (2)$$

A freshly prepared calcium nitrate solution (of 0.99 M) was synthesized by calcination of appropriate amount of $CaCO_3$ at 1100 °C in a flow of dry air to obtain anhydrous CaO solid fractions, which was then slurred into 40 mL of 1.99 M nitric acid solution at 85 °C with mechanical stirring rate of 300 rpm.

A proper amount of $Ce(SO_4)_2$ (0.0493 g) was dissolved in 10 mL of 2.3 M HNO_3 and then added drop wisely at a rate of about 0.5 mL/min to $Ca(NO_3)_2$ solution forming a mixture of 0.8 M $Ca(NO_3)_2$ and 3.2×10^{-3} M $[Ce(NO_3)_6]^{2-}$ with fixed cerium fraction with respect to starting metal atomic ratios, Ce/(Ca + Ce), of 0.004 [30]. The as prepared mixture was finally adjusted at pH 8.0 using concentrated ammonium hydroxide, i.e., never exceeding pH 8.0 to almost keep the produced ceric hydroxide in barely soluble form [31].

Afterwards, thirty millilitres of 0.8 M $(NH_4)H_2PO_4$ solution was drop wised with a rate of 0.2 mL/min onto fifty millilitres of the as prepared mixture under vigorous stirring at 85 °C and N_2 purging to prevent probability of phosphated groups substitution by carbonated ions, possibly formed by dissolution of CO_2 gas during the producing process [32]. The pH value of the resultant suspension was maintained at 8.0 all over the reaction progression using concentrated ammonium hydroxide. The produced slurry was finally suspended in 100 mL aqueous solution (containing 1.8 M NH_4OH) and kept at 85 °C for a further 48 h under vigorous stirring with N_2 gas blowing. After the aging, a pure, well-crystallized nanoceria- containing hydroxyapatite precipitate was filtered off and washed several times by sodium acetate solution and deionized water to ensure elimination of any ammonium or nitrate or sulphate residues from the formed powder and ascertain its neutralization. Finally, the produced fine powder was dried at 80 °C for 48 h and calcined at 200 °C for 4 h in dynamic air, and finally debated by Ce@HAp. As a mother solid, calcium hydroxyapatite (HAp) was prepared