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Enhancement of 5-aminolevulinic acid phototoxicity by encapsulation in polysaccharides based nanocomplexes for photodynamic therapy application

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ABSTRACT

Polysaccharides based nanocomplexes have been developed for encapsulation, controlled delivery and to enhance the phototoxicity of the photosensitizer 5-aminolevulinic acid for application in photodynamic therapy. The nanocomplexes were prepared by coacervation in a solvent free environment using chitosan as polycation while alginic and polygalacturonic acid as polyanions. The complexes showed average dimension in the range 90–120 nm, good stability in simulated physiological media and high drug encapsulation efficiency, up to 800 μg per mg of carrier. Release studies demonstrate the possibility to tune the overall release rate and the intensity of the initial burst by changing the external pH. Cytotoxicity and photocytotoxicity tests confirmed the not toxicity of the used polysaccharides. Cell viability results confirmed the improvement of 5-aminolevulinic acid phototoxicity when loaded into the carrier compared to the free form. No effect of the irradiation on the nanocomplexes structure and on the release kinetics of the drug was observed. The results demonstrate that the prepared formulations have suitable properties for future application in photodynamic therapy and to ameliorate the therapeutic efficacy and overcome the side-effects related to the use of the photosensitizer 5-aminolevulinic acid.

1. Introduction

Photodynamic therapy (PDT) is an effective treatment for human premalignant and malignant lesions because it is noninvasive, well tolerated by patients and can be performed repeatedly without cumulative side effects [1,2]. It involves the use of a class of molecules (photosensitizers, PS) which are accumulated in the cancerous tissues and activated by light at specific wavelengths [2,3]. The general profile of PDT treatment is resumed as follow: i) injection of an appropriate dose of PS into the patient bloodstream; ii) accumulation of the PS at the tumour site and iii) irradiation of the tumour site by a light at the specific wavelength. The photodynamic action initiate by the absorption of a photon by the PS followed by a cascade of competitive radiative and non-radiative reactions which result in the oxidation and degradation of vital biomolecules leading to the tumour cell death [4]. The photophysical process of PDT is resumed in the scheme in Fig. 1. Quenching mechanisms of the T_1 state of the sensitizer can be distinguished in two types of reactions [3]; type I which involves electron or hydrogen atom transfer between the T_1 sensitizer and the substrate molecules and type II, based on the interaction between electronically excited triplet in the sensitizer and the ground-state molecular oxygen ($^3O_2$). Energy transmission yield chemically highly active singlet oxygen ($^1O_2$) which is highly reactive towards many biological molecules including lipids, proteins, nucleic acid, leading to cancer cell death [4–7].

The PS for PDT has to meet several criteria: i) chemical stability; ii) water-solubility; iii) high quantum yield of $^1O_2$ generation; iii) no cytotoxicity in absence of irradiation; iv) tumour selectivity; v) accumulation in the target tumour; vi) rapid clearance; vii) high molar absorption coefficient at long wavelength (600–800 nm) that can penetrate deeper tissues [6,7].

Nowadays, there are several PS available for PDT, but one of the most effective is 5-aminolevulinic acid (5-ALA) [2,3]. 5-aminolevulinic acid is a natural biochemical precursor of heme that is metabolised in a series of enzymatic reactions to fluorescent porphyrins, particularly PPIX. The 5-ALA synthesis is regulated by an intracellular pool of free heme via a negative feedback mechanism. Administration of excess exogenous 5-ALA avoids the negative feedback control, and accumulation of PPIX occurs in the target tissue [7]. Systemic administration of 5-ALA results in an overload of the cellular porphyrin metabolism and accumulation of PPIX in various epithelial and cancer tissues. Malignant glioma tissue has been demonstrated to synthesize and accumulate

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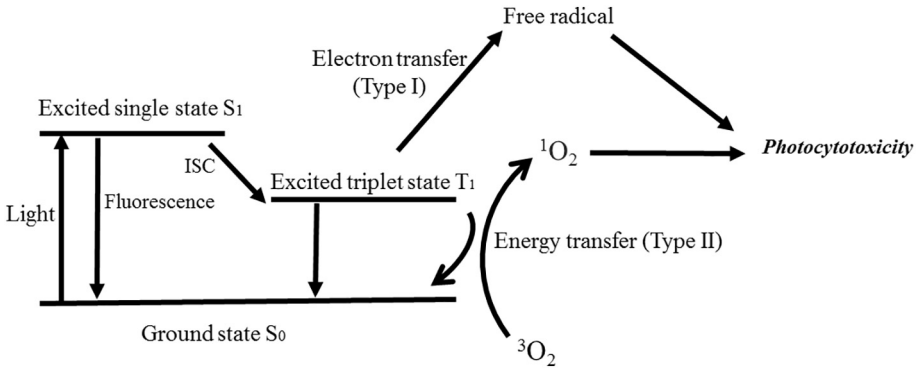

Fig. 1. Schematic illustration of excited states and reactive oxygen species (ROS) [4].

porphyrins in response to 5-ALA administration. The phenomenon of PPIX accumulation may be explained by higher 5-ALA uptake into the tumour tissue or an altered pattern of expression or activity of enzymes (e.g. ferrochelatase) involved in haemoglobin biosynthesis in tumour cells [8]. Limitations in the application of 5-ALA are related to its hydrophilicity which limits its penetration through the skin making it not suitable for treatment of deep-skin neoplasias [9]. Moreover, the insolubility of 5-ALA in physiological media makes difficult the systemic administration limiting the application spectra [10]. To overcome such limitations 5-ALA and other PS have been associated with carriers such as liposomes, micro- or nano-particles [10,11].

The use of polymeric nanoparticles as drug carrier considerably improve the treatment of various tumours [12–13]. Polymer-based nanoparticles have the potential to considerably improve the diagnosis and treatment of the tumour. A wide number of natural, semi-synthetic and synthetic polymers have been prepared, characterised and used to prepare nanocarrier, and a large number of bioactive molecules have been chemically linked or physically loaded.

Chitosan (CS), a polysaccharide obtained by partial deacetylation of chitin, has witnessed an increase interest as a drug delivery carrier due to its intrinsic properties such as biodegradability, biocompatibility, nontoxicity, non-immunogenic, non-carcinogenic, and antibacterial [14,15]. Furthermore, the presence of free amino groups make CS positively charged in mild acidic environment [15]. The most important feature of CS is that it is soluble in water at pH < 6.5 and can easily form nanoparticles by crosslinking with a wide number or agents like glutaraldehyde, tripolyphosphate and several synthetic and natural polyanions like alginic and polygalacturonic acid [16].

ALG is a water-soluble polysaccharide made of α L-guluronic (G) and D-Mannuronic (M) block, which are arranged in an irregular block-wise pattern or varying proportions of G-G, M-G and M-M [17]. CS-ALG complexes result from strong electrostatic interactions between the CS amino groups and ALG carboxylic groups. The past few years have witnessed an increase in the interest on the encapsulation of low-molecular-weight drugs like antineoplastic [18], oculars [19], pulmonary and anti-inflammatory into polymeric and polysaccharide based systems [20–21]. High-molecular-weight molecules, in particular peptides, proteins and nucleotides have been successfully encapsulated in CS-ALG based nanoparticles. Conversely, complexes with PGA are not widely described as the former in the drug delivery field but more has been reported in tissue engineering, in particular for bone reconstruction [22].

However, in vitro studies have reported the high resistance of the CS-PGA complexes to enzymatic hydrolysis and promising results for controlled delivery in the GI tract [22].

The presented work is focused on the use of known polysaccharides (ALG, PGA, CS) to obtain stable nanocarrier to load and improve the efficacy of the photosensitizer 5-ALA for application in PDT. CS-PGA and CS-ALG nanoparticles loaded with 5-ALA were prepared by coacervation method and characterised in terms of average dimension, ζ-potential and morphology. The effect of the weight ratio between the polysaccharides on the capacity to load and release 5-ALA in simulated physiological media was evaluated. Satisfactory results were recorded regarding the diameter and ζ-potential of the nanoparticles, as well as for high encapsulation efficiency, protection of the loaded drugs and sustained release, suggesting the potential application. The phototoxicity of free 5-ALA compared to 5-ALA loaded in the nanoparticles, to prove the effectiveness of the carriers, has been evaluated in HeLa cells line. Cells viability clearly demonstrate the positive outcomes in 5-ALA cytotoxicity when loaded in the prepared systems and irradiated with an appropriate light with established time.

2. Materials and Methods

2.1. Materials

CS (Sigma-Aldrich 20–300 cP, 1 wt% in 1% acetic acid (25 °C, 75–85% deacetylation); alginic acid sodium salt (15–20 cP, 1% in H_2O, M_w 25,000 g/mol); polygalacturonic acid sodium salt from oranges (M_w 50,000 g/mol. > 85% titration); 5-aminolevulinic acid hydrochloride were supplied by Sigma Aldrich. Sodium hydroxide, sodium phosphate, potassium phosphate and potassium hydroxide were bought from IPL Lukes, Uhresky Brod, Czech Republic. Acetic Acid CH_3CO_2H (HPLC grade) was purchased from Chromspec, Brno, Czech Republic.

2.2. Methods

2.2.1. Preparation and Characterization of 5-ALA Loaded Nanocomplexes

CS-PGA and CS-ALG based nanocomplexes were prepared by complexation method using different polycation to polyanion weight ratio (ranging from 0.5 to 5). Briefly, CS was dissolved in aqueous solution (1% v/v acetic acid) while ALG and PGA were dissolved separately in mild alkaline aqueous solution. All prepared solutions were accurately filtered (pore size 0.45 μm) to remove any residue and dust. 5-ALA was dissolved in distilled water at 1 mg/ml concentration. Once complete dissolution, the solution containing 5-ALA (V = 1 ml) was mixed with CS solution and left under stirring for 1 h in dark and at room temperature (22 °C ± 1 °C). Subsequently, under vigorous stirring, established volume of ALG or PGA was added dropwise to CS + 5-ALA mixture and stirred for 30 min. The dimensions and ζ-potential of the obtained nanoparticles were measured by dynamic light scattering (Nano-ZS Zetasizer, Malvern Instruments) at 25 °C and 173° backscattering. Before measurement, the suspension was sonicated in an ultrasound bath (50 MHz) for 20 min. All measurements were performed in triplicate.

The amount of 5-ALA loaded into CS-ALG and CS-PGA nanoparticles was evaluated by UV-VIS spectrophotometer at 264 nm (Perkins-Cary 300 Ultraviolet-Visible) by subtracting the 5-ALA content from the supernatant. A standard curve, prepared by measuring the intensity of absorption at the known concentration of 5-ALA and the encapsulation efficiency (EE) was calculated by the following equation: