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Matrices of a hydrophobically functionalized hyaluronic acid derivative for the locoregional tumour treatment

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ABSTRACT

A hyaluronic acid (HA) derivative bearing octadecylamine and acylhydrazine functionalities has been here employed for the production of a paclitaxel delivering matrix for locoregional chemotherapy. Through a strategy consisting in a powder compression and a plasticization with a mixture water/ethanol, a physically assembled biomaterial, stable in solutions with physiologic ionic strengths, has been produced. Two different drug loading strategies have been adopted, by using paclitaxel as chemotherapic agent, and obtained samples have been assayed in terms of release in enhanced solubility conditions and in vitro and in vivo tumoural cytotoxicity. In particular sample with the best releasing characteristics was chosen for an in vivo evaluation against a HCT-116 xenograft on mice. Local tumour establishment and metastatic diffusion was assayed locally at the site of xenograft implantation and at the tributary lymph nodes. Obtained results demonstrated how loading procedure influenced paclitaxel crystallinity into the matrix and consequently drug diffusion and its cytoreductive potential. Chosen paclitaxel loaded matrix was able to drastically inhibit HCT-116 establishment and metastatic diffusion.

1. Introduction

The locoregional administration of chemotherapeutic agents for the treatment of tumour progression can be a reasonable strategy to delay tumour recurrence after surgical debulking. Ovarian, colon and breast cancers are some examples of regional tumours routinely treated by a first surgical resection followed by systemic chemotherapy [1]. The efficiency of surgical debulking, and the subsequent chemotherapy or radiotherapy influence the outcome of tumour treatment. However the efficacy of chemotherapy is often limited by a poor drug bioavailability and high toxicity. The locoregional administration of chemotherapeutic agents can be an efficient strategy to reduce drug toxicity, to improve its bioavailability and to inhibit growth of micro lesions not removed by surgery [2,3]. To prolong the in situ drug permanence and to reduce systemic absorption, thus improving local chemotherapeutic efficacy over time, a drug delivery system, often composed of a drug containing polymeric biomaterial, could be employed.

Essentially, required properties for a polymeric material are: (a) ability to entrap a suitable amount of drug and to control its release so as to allow diffusion and penetration into the tissues near the surgical resection margins, (b) biocompatibility and biodegradability to avoid a surgical intervention to remove the material, (c) appropriate shape and flexibility to allow an easier fitting with tissues [2,3].

Devices already proposed are rigid biodegradable polymeric systems made of polyanhydrides [4,5] or polyesters [6], flexible materials suitable to cover tissues with irregular shape or gel forming solutions able to form viscous fluids when injected into the target tissue (Oncogel) [7–8]. However new materials having appropriate characteristics are necessary to obtain devices that can better satisfy pharmaceutical needs. Hyaluronic acid (HA), a glycosaminoglycan (GAG) of the extracellular matrix, that regulates several tumour signaling pathways [9], can be an appropriate material for the production of drug delivery systems for locoregional tumour treatment [10]. However hydrophilic polymers, like HA, need a crosslinking to retard their dissolution and to better control drug release. As an example, a chemically crosslinked HA hydrogel has been proposed as gel forming device to entrap

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paclitaxel and tested both in vitro and in vivo [11]. Alternatively, an appropriate chemical functionalization can be designed to modify HA properties in order to increase its stability without a chemical crosslinking. For example, the grafting of hydrophobic chains into HA backbone could allow the production of amphiphilic derivatives that may self assemble in aqueous medium forming stable physical hydrogels with potential application for drug delivery or tissue engineering [12,13]. At this aim, polymers such as polyesters (polylactic acid, poly(lactic-co-glycolic) acid, polycaprolactone), or aliphatic chains have been grafted to HA [14–16]. As an example, Pellettier and coll. [17] have proposed amphiphilic HA graft derivatives obtained by linking to the HA backbone short alkyl chains constituted by 12–18 CH_2. Derivatives HA-C_{12} and HA-C_{18} obtained with a derivatization degree equal to 1.5–8 mol% (alkyl chains for 100 HA repetitive units) showed a strong associative behavior in aqueous diluted solutions. Novozymes has patented a chemical procedure concerning the grafting of alkyl succinate anhydrides to HA sodium salt in alkaline water [12].

Physical hydrogels prepared by HA hydrophobic derivatives can be used as drug delivery systems where hydrophobic coils can be exploited to increase affinity towards lipophilic drugs, thus improving both drug loading and its release. Recently we have proposed a HA derivative bearing octadecylamine and hydrazido functionalities, to produce hydrophobically assembled biomaterials for regenerative medicine applications [18]. In this work, the presence of hydrophobic assembled coils has been exploited to load paclitaxel, a lipophilic chemotherapeutic agent. A physical hydrogel has been produced as a plasticized matrix and characterized by evaluating its stability in media with different ionic strength as a function of time or in the presence of hyaluronidase. In vitro experiments have been performed to evaluate the release of paclitaxel (PTX) from samples obtained using different procedures for drug loading. Moreover, drug delivery profiles and cell toxicity using human colorectal tumour cells (HCT-116) have been related to the effects of drug loading procedure on PTX solid state. Finally an in vivo test has been performed on human colorectal tumour cells (HCT-116) xenograft model on athymic nude mice (Foxn1^{nu}) to evaluate the ability of a chosen drug loaded matrix, to inhibit nodule formation, as well as tumour growth and metastasis occurrence.

2. Experimental

2.1. Materials and methods

Hyaluronic acid (2000 kDa) was from Altergon (Italy). Low molecular weight HA (Mw 220 kDa, polydispersity index 1.8) and the tetrabutylammonium salt of hyaluronic acid (HA-TBA) were produced as reported elsewhere [19]. Octadecylamine, tetrabutylammonium hydroxide (TBA-OH), testicular hyaluronidase (HAase) (1040 U/mg), bis(4-nitrophenyl) carbonate (4-NPBC), picrylsuphonic acid solution (2,4,6-trinitrobenzenesulfonic acid-TNBS), *t*-butyl-carbazate and hexamethyldisilazane were purchased from Sigma–Aldrich (Milano, Italy). Hydrazine monohydrate, anhydrous dimethylsulfoxide (DMSO) were from Fluka (Milano, Italy). Paclitaxel was purchased from VWR (Milano, Italy).

^{1}H-NMR spectra were obtained with a Brucker AC-300 instrument of 300 MHz. Size exclusion chromatography (SEC) was performed using a multidetector SEC system equipped with a Water 600 pump, a Water 410 Refractive Index Detector and a Linear column from Water (particle size 5 μm). The calibration curve was determined by using standards of HA purchased from Hyalose (USA). The elution medium was 200 mM phosphate buffer (pH 6.5)/MeOH 90:10 (v/v), with a flow rate 0.6 ml/min at 35 °C.

High Pressure Liquid Chromatography (HPLC) for paclitaxel quantification was performed employing a Agilent 1100 instrument equipped with an UV detector and a Phenogel column employing water/acetonitrile 1/1 as mobile phase, a flux equal to 0.8 ml/min and a λ of 226 nm.

UV measurements were carried out by using a Shimadzu UV-2401PC spectrophotometer.

Scanning electron microscopy (SEM) images were recorded by using a scanning electron microscope Philips XL30 ESEM.

2.2. Synthesis of Hy-HA-C_{18} derivative

1 g of HA-TBA was dissolved in 90 ml of DMSO anhydrous at 40 °C, then 10 ml of DMSO containing 340 mg of 4-NPBC were added to obtain a molar ratio 4-NPBC/HA-TBA repetitive units equal to 0.7. The reaction was maintained at this temperature for 4 h [20]. After this time the appropriate amount of octadecylamine (C_{18}) was added to obtain a molar ratio octadecylamine/4-NPBC equal to 0.7, then the temperature was set at 60 °C and after 24 h, it was decreased at 40 °C. Therefore, an appropriate amount of hydrazine monohydrate (Hy) was added to obtain a molar ratio Hy/4-NPBC equal to 10. After 1 h, the obtained suspension was filtered, then NaCl saturated solution (1 ml for gram of polymer) was added and the sample was precipitated in acetone. The obtained Hy-HA-C_{18} derivative was washed several times with hot ethylacetate, then with a mixture acetone/water 80:20 and finally with acetone and dried under vacuum. The yield of Hy-HA-C_{18} derivative was 75% w/w with respect to the starting HA-TBA.

The reaction was performed in triplicate and the derivatization degree in C_{18} and Hy, expressed as mol%, were detected via ^{1}H-NMR and colorimetric assay, respectively.

A typical ^{1}H-NMR spectrum of Hy-HA-C_{18} derivative showed: δH (300 MHz; D_2O/$THFd_8$ 1:1) 0.99 (3H, s of the–CH_3 of the octadecylamine chain), 1.5 (32H, br m, of the –CH_2–$(CH_2)_{16}$–CH_3 of the octadecylamine chain), 1.9 (3H, s, of the –CH_3 of the N-acetylglucosamine portion of HA); 3.3–4.0 (pyranosyl groups of HA).The derivatization degree in C_{18} was calculated by comparing the integral of peaks at δ 0.99 and δ 1.5 due to the octadecylamine chain with the integral of the peak at δ 1.9 due to HA. The derivatization degree in Hy was calculated by TNBS colorimetric assay using t-butylcarbazate as a standard, as reported elsewhere [21].

2.3. Production of empty and drug loaded Hy-HA-C_{18} matrices

Empty and paclitaxel loaded Hy-HA-C_{18} matrices were produced in two steps: (1) compression of polymer alone or polymer plus drug to produce tablets with a diameter of 1 cm; (2) plasticization of the obtained tablets.

In particular, for the production of empty matrix, 25 mg of Hy-HA-C_{18} powder were used to fill a cylindrical mold and compressed at 2000 kg/cm^2 for 30 s. For the production of drug loaded matrices, two general procedures were used: (*procedure a*) – the polymer powder was drop-wise wetted with 100 μl of acetone containing 1 mg of paclitaxel and left to dry under a laminar flux hood, then dried powder was compressed and plasticized (*sample a*); (*procedure b*) – polymer and paclitaxel powders were mixed and directly compressed, then plasticized (*sample b*). For both procedures, the ratio Hy-HA-C_{18}/PTX was 25/1 w/w.

In all cases, for plasticization, tablets were transferred into a plastic mold with a diameter of 1 cm and treated with 300 μl of a mixture water/ethanol 2:1. After 4 h, the plasticized tablets with a diameter of 1 cm and a height of 1 mm were dried under vacuum. The amount of paclitaxel loaded into plasticized tablets (matrices) was evaluated selecting 3 different samples that were cut in 4 little pieces. Each portion was weighed then dissolved in 6 ml of a