International Journal of Pharmaceutics 474 (2014) 91–94

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Rapid communication

Folate receptor targeted self-assembled chitosan-based nanoparticles for SPECT/CT imaging: Demonstrating a preclinical proof of concept

András Polyák [a,*], István Hajdu [b], Magdolna Bodnár [b], Gabriella Dabasi [c], Róbert P. Jóba [c], János Borbély [b], Lajos Balogh [a]

[a] National "F.J.C." Research Institute for Radiobiology and Radiohygiene (NRIRR), Budapest,Hungary
[b] BBS Nanotechnology Ltd., Debrecen, Hungary
[c] Department of Nuclear Medicine, Semmelweis University, Budapest, Hungary

ARTICLE INFO

Article history:
Received 23 April 2014
Received in revised form 23 July 2014
Accepted 31 July 2014
Available online 2 August 2014

Keywords:
Chitosan
Nanoparticles
Nanomedicine
SPECT-CT
Folate receptor
^{99m}Tc

ABSTRACT

A new biocompatible, biodegradable, self-assembled chitosan-based nanoparticulate product was successfully synthesized and radiolabeled with technetium-99m, and studied as a potential new SPECT or SPECT/CT imaging agent for diagnosis of folate receptor overexpressing tumors. In the present study we examined the conditions of a preclinical application of this labeled nanosystem in early diagnosis of spontaneously diseased veterinary patient using a human SPECT/CT device. The results confirmed that the nanoparticles accumulated in tumor cells overexpressing folate receptors, contrast agent revealed higher uptake in the tumor for a long time. Preclinical trials verified that the new nanoparticles are able to detect folate-receptor-overexpressing tumors in spontaneously diseased animal models with enhanced contrast.

1. Background

Electrostatic interactions between oppositely charged biopolymers can result stable self-assembled particulate systems by the interaction between the functional groups (Vasiliu et al., 2005; Liu et al., 2005; Reihs et al., 2004; Noble et al., 1999; Feng et al., 2005). Compared the low molecular weight SPECT radiopharmaceuticals, nanoparticle based compounds can circulate in the body for longer time, and target the specific studied (cancer) cells with higher efficiency by active or passive mechanism (Garcia-Bennett et al., 2011; Haley and Frenkel, 2008; Byrne et al., 2008). The nano-sized particles can easily penetrate cellular membranes due to their relative small size, allowing them to act contrast agent carriers. Many reviews have been published on polyelectrolyte complexes containing chitosan (Vasiliu et al., 2005; Liu et al., 2005; Reihs et al., 2004). Chitosan-based nanoparticle systems have special set of properties, such as low toxicity, biocompatibility, biodegradability, low immunogenicity, and antibacterial properties.

A potential specific target ligand is the vitamin folic acid (FA) for targeting the cell membrane folate receptors (FR). The possibility of using folate receptor as a tumor marker first arose in 1991 during the investigation of human ovarian carcinoma cell lines (Coney et al., 1991). Nowadays FR is a valuable molecular target for tumor-selective radionuclide delivery (Ke et al., 2003) and therapy that is in approximately 90% highly expressed on a variety of cancers (Ross et al., 1994; Lu et al., 2011; Müller et al., 2007; Guo et al., 2010) such as ovarian or oral carcinomas. Tumor-specificity of targeting folate receptors can significantly improve the specificity, efficacy and bioavailability of folate-functionalized nanoparticles compared to free drugs or contrast agents in drug delivery or tumor-imaging applications (Garcia-Bennett et al., 2011).

In our latest studies, poly-gamma-glutamic acid (γ-PGA) was utilized to create stable colloid particles by self-assembly with chitosan biopolymers in aqueous media, and folic acid as targeting ligand was conjugated to γ-PGA via amino groups to mediate the cellular uptake of nanoparticles. Synthesis (Hajdu et al., 2008) and investigation as a novel nanoscale carrier system (Keresztessy et al., 2009) were published. The stable nanoparticles were characterized by several physicochemical methods, which demonstrated that the nanosystem consists of separated spherical particles in an aqueous environment and also in dried state (Hajdu et al., 2008; Keresztessy et al., 2009). The whole-body biodistribution, the intracellular localization and toxicity of nanoparticles have also been studied (Keresztessy et al., 2009). Cellular internalization of nanoparticles was tested with the folate-receptor-overexpressing A2780/AD ovarian cancer cells, and

* Corresponding author.
E-mail address: bandi.polyak@gmail.com (A. Polyák).

http://dx.doi.org/10.1016/j.ijpharm.2014.07.055
0378-5173/

they were non-toxic when tested either on cell cultures or in animal feeding experiments (Keresztessy et al., 2009). First biodistribution values were determined with HeDe (Hepatocarcinoma Debreceniensis) tumor cell line transplanted Fischer 344 rat models in previous studies (Polyák et al., 2013). In the laboratory animals, a significant higher uptake (about 9% of total injected dose) was observed in the HeDe tumor-transplanted left kidneys compared to the right normal kidney activities and compared to the resulted maximum tumor-uptakes (about 2% if I.D.) of the ^{99m}Tc-DMSA-injected control animals (Polyák et al., 2013).

In this present study we examined the conditions of the first real clinical (veterinarian) application of the radiolabeled nanosystem in order for early diagnosis of a spontaneously diseased cat patient's oral tumor using a human SPECT/CT device. Veterinary animal patients with spontaneous occurred tumors share many environmental risk factors with their human owners, suggesting that they are valuable and ideal animal models in preclinical tests (Hansen, 2004; Kelsey et al., 1998).

2. Methods

2.1. Chitosan-based nanoparticles for SPECT/CT imaging

Stable self-assembled nanoparticles were developed via an ionotropic gelation process between PGA-FA and chitosan. Chitosan solution (c = 0.3 mg/ml, V = 1 ml, pH 4.0) was added into PGA-FA solution (c = 0.3 mg/ml, V = 2 ml, pH 9.0) under continuous stirring. An opaque aqueous colloidal system was gained, which remained stable at physiological pH. Folic acid was conjugated via the amino group to poly-γ-glutamic acid using the carbodiimide (EDC) technique. Chitosan (CH; degree of deacetylation = 88%, M_w = 320 kDa) was purchased from Sigma–Aldrich Co., Hungary. Poly-γ-glutamic acid (PGA; M_w = 400 kDa) was purchased from Vedan Group, Taiwan. In our previous works, complete physico-chemical characterization of the new nano-compound was performed (Hajdu et al., 2008; Keresztessy et al., 2009). The hydrodynamic size and polydispersity of nanoparticle compound were determined by means of dynamic light scattering (DLS) technique using a Zetasizer Nano ZS (Malvern Instruments Ltd., UK). Mean diameter of the labeled product was 124 nm. The labeling method with the generator product [^{99m}Tc] technetium was also described earlier (Polyák et al., 2013). The process proved to be relatively simple, rapid and reproducible using stannous chloride as reducing agent and thin layer chromatography (ITLC-SG with methyl ethyl ketone) for determination of labeling efficiency.

2.2. Cat patient with spontaneously occurred oral tumor

A spontaneously diseased cat was studied by applying the new ^{99m}Tc-labeled nanoparticles and using a human SPECT/CT camera (Anyscan, Mediso Inc., Hungary). The 12 years old, spayed female cat (body weight: 4450 g) was referred to our institute with a history of recurring oral malignancy. The local surgery was performed by the first line veterinary four months earlier. Routine histopathological examination (HE stains) proved the presence of oral carcinoma with no free surgical margins and the immunohistochemical examination revealed the folate-receptor overexpressing property of the sample. Two months post surgery a local, fast-growing recidiva was seen at the operation site. At the time of our SPECT/CT examination the non-ulcerous tumor showed 28 mm × 34 mm × 39 mm extensions measured by a calipper.

Immediatelly after radiopharmaceutical application (500 MBq/2 ml intravenously) anesthesia (isoflurane inhalation) was induced then 5–120 min post application serial nuclear medicine imaging (whole body, regional static and SPECT) and whole body CT imaging was carried-out. Image fusion and quantitative analysis (heart, liver, kidney, urinary bladder and tumor uptakes) of radiolabeled ligand distribution was performed using the dedicated software (Interview, v. 3.0, Mediso, Hungary) of the SPECT/CT equipment.

3. Results

Spontaneously diseased cat model was treated i.v. using the radiolabeled nanoparticles. No clinical side effects in experimental animal (screaming, salivation, tremor, hypo- or hyperdyspnoea, diarrhea, restlessness or comatose state) were recorded during the entire examinations and the following 24 h. Hematological and biochemical panel from the blood-samples before the examination and 24 h post-examination did not show a difference.

In early whole-body scans uptake by the heart, large blood vessels, liver, spleen and kidneys and a moderate uptake by the bone marrow were visualized. The heart, the liver and the kidneys with the urinary tract showed the highest activity and slow elimination to 2 h biodistribution values. Two hours after injection, a RES-biodistribution could be observed (Table 1). A slow increase was observed in the oral tumor uptake in the first 2 h up to 3.11% accumulation of total injected activity.

Besides, only a very low uptake of radiolabeled agent was measurable and visible in the thyroid and the lungs, which indicated that the compound had proper labeling efficiency and high particle size stability after injection. The negligible uptake of thyroids indicated low presence of non-bound pertechnetate, and the low observed activities in the lungs showed that lung capillaries could not accumulate any radiolabeled particulates above one micrometer size.

An exactly definable and clear tumor uptake could be observed in the SPECT/CT images taken 2 h after injection by three-dimensional mapping of the feline patient's head region (Figs. 1 and 2). Brighter color indicates higher isotope activity (the white tone represents maximum detectable activity). Highest tumor uptake was verifiable in slices of Fig. 3.

4. Discussion

In our previous papers (Hajdu et al., 2008; Keresztessy et al., 2009; Polyák et al., 2013) we published the radiolabeling method, *in vitro* quality control data of this same self-assembled radiolabeled chitosan-based nanoparticulates and we also proved the retained targeting capabilities of radiolabeled nanoparticle in a rat model *in vivo*. Now in this present study we proved the potential of this radiolabeled ligand in a larger spontaneously occurring diseased animal model using a human SPECT/CT device. This novel ^{99m}Tc-radiolabeled folate receptor targeting nanocompound proved to be a safe and reliable radiopharmaceutical-applicant. Compared to previously published biodistribution results of independent studies (Ke et al., 2003; Lu et al., 2011), the whole body distribution of labeled compound showed similar colloid-distribution in our animal model with relative high uptakes in the RES-organs (liver, spleen). However, the relative high tumor-uptake revealed high quality scans from the folate overexpressing tumor. Further studies need to be performed to elucidate the ideal imaging-time after application and the tumor targetting scale in animal models and in human patients.

Table 1
Organ activity percentages 2 h post injection using ^{99m}Tc labeled nanosystem.

Liver	35.76%
Kidneys	8.85%
Bladder	1.36%
Tumor	3.11%