Development of human albumin-based nanoparticles for diagnostic optical molecular imaging of early inflammation and adenocarcinoma

Alshaimaa A. Abdelmoez1,2, Gudrun C. Thurner1, and Paul Debbage1

1Innsbruck Medical University, Department of Anatomy, Histology, and Embryology, Innsbruck, Austria, and 2Assiut University, Faculty of Pharmacy, Department of Pharmaceutical Organic Chemistry, Assiut, Egypt

Introduction

Nanoparticle technology has potential as a tool for the development of “Trojan horses” which can steer payloads consisting of hundreds of imaging signal enhancers which accumulate in a specific body locations thus enhancing optical imaging for diagnostic purposes. Serum albumin, a natural circulating multifunctional protein which transports hormones, vitamins, drugs, ions, and other plasma constituents in the circulation, is an excellent candidate carrier [1]. Various types of proliferating tumors, and also inflamed cells, accumulate and utilize albumin as a major energy and nitrogen source for protein synthesis and repair. Therefore human serum albumin (HSA) is in principle a non-toxic, non-immunogenic and easily biodegradable excipient for targeted delivery to sites of inflammation or to cancer cells. In several years of previous work, we demonstrated that our albumin-based nanoparticles can be targeted specifically to a target molecule and can serve as a molecular imaging tool for MRI in vivo [2, 3, 4]. In view of clinically licensed nanoparticle-based therapeutics, for example the nanoparticle albumin bound technology (Nab-technology) for targeted drug delivery of paclitaxel (Abraxane®), that reached the market and was approved by the FDA for treatment of metastatic breast cancer in 2005, metastatic non-small cell lung cancer in 2012, and metastatic pancreatic cancer in 2013 [5], we now aim to apply our experience gained in scientific research to translational research. The work we report here focuses on the development of brightly fluorescent, targeted albumin nanoparticles, designed to image the early stages of inflammation and the development of carcinomas. Since only a small number of blood vessels are expected to express positive biomarkers in the early stages of inflammation, and since early adenocarcinoma lesions may not express them strongly, it is essential to create nanoparticles of intense intrinsic brightness in order to achieve a clinically relevant optical detection systems. This report describes the development of such nanoparticles, to which later a targeting moiety will be added to increase specific uptake at target sites.

Materials and methods

In the present study, fluorescein isothiocyanate (FITC) emitting green light ($\lambda_{\text{ex}}$ 490 nm, $\lambda_{\text{em}}$ 515 nm) was used as a fluorochrome. In addition, a range of red-emitting fluorochromes was compared with FITC. The fluorochrome was attached covalently to albumin to prepare fluorescent conjugates of albumin, and then nanoparticles were prepared from these conjugates by coacervation and crosslinking.

Synthesis

The following analytical-grade chemicals were purchased from Sigma Aldrich (Vienna, Austria) and from commercial sources without further purification: HSA (fraction V), FITC, 1-ethyl-3-[3-(dimethylamino)-propyl] carbodiimide (EDC), N-hydroxysuccinimide...
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(NHS), sodium chloride, activated charcoal, methanol, and hydrochloric acid. Albumin was first defatted and stabilized according to a procedure described in our earlier work [3]. Then stabilized albumin was conjugated to either FITC or to the carbodiimide-activated red fluorochrome. Fluorescent nanoparticles were then prepared by coacervation and cross linking of the corresponding fluorescent conjugates. We varied the synthesis protocols systematically (e.g., molar ratios fluorochrome: albumin, conjugation protocol, inter al.).

Assessment for fluorescence intensity was by use of either fluorescence spectrometry or by use of a custom-designed imaging apparatus mimicking endoscopes presently in routine clinical use. Molar ratios were assayed in MALDI-TOF mass spectrometry. Nanoparticle size and dispersity were characterized by use of PCS and by negative contrast transmission electron microscopy (TEM). Particle mechanical integrity and purity were tested by use of thin layer chromatography (TLC, 1:1:1; v/v; 1M citrate-buffer/acetic acid/water) and SDS-PAGE (Figure 1). Nanoparticle batches were stored in the dark at 4 °C.

Results

The nanoparticles carrying either FITC or a red fluorochrome were detectable in an endoscope simulacrum at fluorochrome concentrations as low as 50 nmol/mL. The largest particles were 160 nm in diameter and the smallest 70 nm, while 80% of them were between 70 and 100 nm as analyzed by PCS; the TEM results correlated with PCS data, as shown in Figure 1A, B. Under TEM, the nanoparticles were spheroidal (Figure 1A).

Figure 1. A: TEM micrograph of negatively contrasted red fluorescent albumin nanoparticles (magnification × 110,000, calibration bar 200 nm); B: PCS measurements of the same batch of nanoparticles; C: TLC sheet of: 1-FITC, 2-HSA, 3-FITC-HSA conjugate, 4-FITC-HSA nanoparticles, viewed in 254 nm UV light; D: the same TLC sheet under a 366 nm UV lamp; E: SDS-PAGE gel of: 1) standard (the visible band is 10 KDa, see scale at left), 2) HSA, 3) FITC-HSA nanoparticles batch #1, 4) FITC-HSA nanoparticles batch #2, 5) FITC-HSA conjugate, 6) red fluorescent nanoparticles.
They typically carried 2 – 10 fluorochromes per albumin molecule as confirmed by MALDI-TOF (data not shown). Calculation based on geometrical considerations and a packing density of 70% indicated that a nanoparticle could comprise a maximum of 1,400 HSA molecules, so each nanoparticle would bear 2,800 – 14,000 fluorochromes. In TLC analysis, no free dye was detected, neither in the conjugate nor in the nanoparticle lanes (Figure 1C, D). The nanoparticles did not disintegrate in sodium dodecyl sulfate and thus did not enter polyacrylamide gels (Figure 1E lanes and 4, 6). They were also stable during storage at 4 °C for several months, with little aggregation.

Discussion

The nanoparticles exhibited adequate fluorescence intensity within the range calculated to make them visible by use of endoscopes in routine clinical use, the calculations being based on the cellular properties of early inflammatory or tumorous lesions. This detectability now requires testing against live tissues. The fluorochrome conjugation to albumin was successful as no free dye was detectable by TLC. The nanoparticles are mechanically robust as they resisted denaturing by SDS. They comprised uniform populations with polydispersity less than 1.0 and showing no or little aggregation.

Conclusion

Based on stability, purity, ease of manipulation, and low poly dispersity index, our nanoparticles are candidates for further development as imaging agents for diagnosis. These results embolden us to evaluate nanoparticle-specific uptake by inflamed or cancer cells in animal models, such as are already available for oncological studies in collaboration with our partners at the University of Erlangen, and we are developing animal models to visualize early inflammation. Attachment of a specific antibody to the nanoparticles to allow their accumulation exclusively at relevant target sites will be our next aim.

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Conflict of interest

The authors declare there is no conflict of interest.

References