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Blood protein coating of gold nanoparticles as potential tool for organ targeting

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ABSTRACT

Nanoparticles (NP) and nanoparticulated drug delivery promise to be the breakthrough for therapy in medicine but raise concerns in terms of nanotoxicity. We present quantitative murine biokinetics assays using polyelectrolyte-multilayer-coated gold NP (AuNP, core diameter 15 and 80 nm; ^{198}Au radiolabeled). Those were stably conjugated either with human serum albumin (alb-AuNP) or apolipoprotein E (apoE-AuNP), prior to intravenous injection. We compare the biokinetics of protein-AuNP-conjugates with citrate-stabilized AuNP (cit-AuNP). Biokinetics was complemented with histology in organs with high AuNP content using 15 nm double fluorescently-labeled alb-AuNP-conjugates. Protein conjugation massively reduced liver retention (alb-AuNP: 52%, apoE-AuNP: 72%, cit-AuNP: >95%, at 19 h and 48 h) when compared to cit-AuNP. The protein conjugates were retained in lungs (alb-AuNP (18%) and spleen (alb-AuNP (16%), apoE-AuNP (21%) at 19 h. Alb-AuNP show significantly increased fractions in lungs (factors: 60 (30 min); 111 (19 h); 235 (48 h) and brain (factors: 70 (30 min); 90 (19 h); >200 (48 h) compared to cit-AuNP (control) - or even to apoE-AuNP. The influence of protein conjugation on the biodistribution disappears for 80 nm AuNP comparing to control. Histologically, the 15 nm alb-AuNP are mainly located in the endothelium of brain, lungs, liver and kidneys after 30 min, while at 19 h they moved deeper into the parenchyma *e.g.* in hippocampus. Our study clearly suggests that stable conjugation of AuNP with albumin and apoE prior to intravenous administration increases specificity and efficiency of NP in diseased target-organs thus suggesting a potential role in nanomedicine and nanopharmacology.

1. Introduction

One of the big promises of nanomedicine, are advancements in optimized and targeted delivery of drugs to diseased tissue without affecting healthy tissue. To guide nanoparticles (NP) to the target the most common strategy is their surface functionalization with specific compounds such as antibodies, nanobodies, small molecules, toxins, and receptor-specific molecules such as insulin, or apolipoprotein [1–3]. Recent studies showed that aspects such as protein corona formation after NP injection [4–10], low tissue or tumor penetration, and specificity to receptors dramatically limit the success of targeted delivery. These studies also indicate that the major benefit from active targeting is the improved endocytosis of drug loaded NP [11].

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The biodistribution of NP is defined as the accumulation of nanoparticles in different organs, tissues and body fluids while biokinetics is a time series of biodistribution. The biodistribution is influenced by several factors such as size, surface charge, and surface functionalization. Recent studies indicate that all these factors are important determinants of the composition of the protein corona binding to NP surfaces in blood [4,12].

In the present study, we investigated the effect of two proteins, human serum albumin (HSA) and apolipoprotein E (apoE) on the quantitative biokinetics of different gold NP (AuNP) conjugates (apoE conjugated AuNP = apoE-AuNP and HSA conjugated AuNP = alb-AuNP), citrate-stabilized AuNP (cit-AuNP) and the corresponding polyelectrolyte coated AuNP by measuring the NP content and concentration in all organs, tissues, blood and the remaining carcass (skin, skeleton, soft tissue) at different time points. We also included in our study the total excretion (feces and urine). A protein terminated polyelectrolyte matrix was self-assembled layer-wise by electrostatic interactions on monodisperse, spherical 15 or 80 nm AuNP. Both proteins (HSA and apoE) are frequently found in the protein corona [4,8,13–16] of NP exposed to blood serum and are supposed to influence the biodistribution.

Albumin is the most abundant blood protein and has several important functions in blood. The high content of albumin maintains the osmotic pressure in blood and tissues. Additionally, albumin is an important transporter molecule for hydrophobic molecules such as hormones and fatty acids [17]. Physiologically, low amounts of albumin are released by kidneys, about 50 times more by intestinal tract, while most albumin circulates in blood or is immobilized temporally in the extracellular space. The majority of extravascular albumin is located in muscles (40%) and skin (41%). Albumin binding receptors have been shown to be distributed on endothelial cells of the vasculature of liver, lungs, skeletal muscles, adipose tissue, and heart since albumin is a transporter of fatty acids [18]. This, however, means that the alb-AuNP are much less phagocytized by macrophages of the RES (reticuloendothelial system) than cit-AuNP and the amount of alb-AuNP in endothelial cells with albumin binding receptors depends on the density of the albumin receptors in the vasculature of the different organs.

ApoE is a protein involved in transport and selective uptake of fatty acid, cholesterol and cholesterol esters in form of mainly low density lipoproteins (LDL).

In a previous work [19] NP localization in brain of mice for up to 7 days has been determined by electrostatic binding of HSA covalently labeled with Cy5.5.

In order to quantify the amount of AuNP in organs we used radioactive ^{198}Au produced by neutron activation of an aqueous suspension of initially prepared citrate-stabilized AuNP in a nuclear research reactor. Radioactively labeled AuNP of similar sizes and surface coatings were already used in previous NP biokinetics studies [15,20–22]. After neutron activation the final steps of protein conjugation with ^{198}AuNP were performed prior to *in vivo* use.

To our knowledge, this study provides for the first time biokinetic and histological data of stable protein-AuNP-conjugates and demonstrates a severely different accumulation and retention pattern in various organs with much less liver accumulation, and significant accumulations in spleen and lungs when compared to the control AuNP with a citrate surface modification. Furthermore, we showed that these protein conjugations lead to increased accumulations in the central nervous system (CNS). Both the change in organ distributions as well as the increased accumulation in the CNS may be beneficial for potential pharmacological applications in the future.

ApoE is, along with apoB100, a ligand which binds to the LDL (low density lipoprotein) receptor with high affinity but also to VLDL (very low density lipoprotein) receptors and Low density lipoprotein *receptor*-related protein 1 [23]. In its function as a transporter for cholesterol in the body it is mainly associated with VLDL and the main target is the liver. The LDL receptor promoting the VLDL uptake is located in clathrin-coated pits. The transport property of apoE for cholesterol required for cell membrane integrity makes the LDL receptor ubiquitous with higher densities in macrophages and in proliferating tissues (cancer). Once the LDL binds to its receptor it remains on the cell surface for <10 min and then by entering into the acidic endocytotic compartments the protein component of LDL are digested in about 60 min [24]. A more specific target for apoE binding is the VLDL receptor which is highly expressed in heart, muscle, adipose tissue and brain and is barely present in the liver, in contrast to the LDL receptors [25]. The VLDL receptor binds exclusively VLDL driven by the apoE and leads to a recycling of apoE in HDL (high density lipoprotein) rather than to lysosomal digest as it was observed for LDL receptors [26].

2. Materials and methods

2.1. Layer-by-layer functionalization of gold nanoparticles

Monodisperse, spherical 15 ± 2 nm (TEM determined) AuNP were prepared by the method of Turkevich of gold citrate reduction [27] from an aqueous solution, and gold (Au) radioactively labeled by neutron irradiation (half-life: 2.7 days) in a nuclear research reactor (1 mL with 1 mg of AuNP yielding a specific radioactivity of 10–20 MBq/mg). Subsequently the AuNP were coated with polyelectrolytes and proteins using a modified procedure described before [19,28]. The coating was analyzed in detail by SERS (surface enhanced Raman spectroscopy) [29] and a qualitative biodistribution study showed accumulation of the NP in defined brain areas of healthy mice [19].

In brief, for the HSA conjugation, a polystyrene sulfonate (PSS) layer was first deposited by adding drop wise 1 ml AuNP solution of 70 μg/mL of Au concentration to a PSS solution (4.3 kDa; 10 mg/ml; 200 μl) under constant mixing. This was followed by 20 min of incubation in the dark and two washing steps with Milli-Q grad water. For the second layer, the PSS coated AuNP were added drop wise to 500 μl of a 1:1 mixture of HSA (1 mg/mL) and poly-allylamine hydrochloride (PAH) (1 mg/mL). In the following the resulting NP are called alb-AuNP. After incubation the coated alb-AuNP were washed once. Finally, the coated alb-AuNP were concentrated to a final concentration of about 500 μg alb-AuNP per mL suspension by centrifugation.

In the case of apoE functionalization, the layer sequence was changed. The different sequence for apoE was necessary to prevent NP agglomeration during particle coating. The first layer was deposited by adding drop wise 1 mL of AuNP (70 μg/mL) to PAH (15 kDa; 3 mg/mL; 500 μl) solution. The incubation and washing was performed as described in the previous paragraph. Then a second layer was deposited by adding the coated NP to 200 μl of PSS (10 mg/mL) solution. After incubation in the dark for 20 min and three washing steps the coated NP were concentrated from 10 mL to 6 mL which were added drop-wise to 2 mL of apoE (100 μg/mL) in Milli-Q water. After incubation in the dark and one washing the coated NP were concentrated by centrifugation to a final concentration of about 500 μg AuNP per mL suspension. These NP are called apoE-AuNP conjugates.

For comparison, cit-AuNP (~500 mg/mL) as well as AuNP without proteins but with the same polyelectrolyte coating (1st layer: PSS; 2nd layer: PAH = 1s2p-AuNP; ~500 mg/mL) and 1st layer: PAH; 2nd layer: PSS = 1p2s-AuNP; ~500 mg/mL) were used for the biokinetics studies. The polyelectrolyte coated AuNP were prepared as described above. Additionally, commercially available pure 80 nm AuNP from BBI Life Sciences (Cardiff, UK) were coated as described above with the polyelectrolyte alone or a coating containing either apoE or HSA. Own TEM images of the 80 nm Au core NP confirmed the size and narrow size distribution.

For the *in vivo* imaging experiments we prepared fluorescently labeled coated NP. Instead of PAH we used FITC (fluorescein isothiocyanate)-PAH (Sigma, Milan; Italy) and HSA was covalently bound to Cy5.5 following the procedure described below.

In all described coating procedures the progress of the coating was followed and confirmed by zeta potential (ζ) and dynamic light scattering measurements with Malvern Nano-ZS instrument (Malvern, UK). The monodispersity was determined from the polydispersity index. The results are comparable to those already published [19,28,29].

2.1.1. Fluorescently labeled coated AuNP for histology

The preparation of the fluorescently labeled AuNP for the histology experiments followed the protocols described in detail in Sousa et al. [19] In brief, for the polyelectrolyte coating a commercially available 15 kDa FITC-PAH (Sigma–Aldrich, Milan, Italy) was used.

Albumin was covalently bound to the NIR dye Cy5.5. One mg FITC dissolved in 200 μL of dimethyl sulfoxide was added drop-wise to 4 mg/mL of HSA dissolved in