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Nanobody conjugated PLGA nanoparticles for active targeting of African Trypanosomiasis

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ABSTRACT

Targeted delivery of therapeutics is an alternative approach for the selective treatment of infectious diseases. The surface of African trypanosomes, the causative agents of African trypanosomiasis, is covered by a surface coat consisting of a single variant surface glycoprotein, termed VSG. This coat is recycled by endocytosis at a very high speed, making the trypanosome surface an excellent target for the delivery of trypanocidal drugs. Here, we report the design of a drug nanocarrier based on poly ethylen glycol (PEG) covalently attached (PEGylated) to poly(D,L-lactide-co-glycolide acid) (PLGA) to generate PEGylated PLGA nanoparticles. This nanocarrier was coupled to a single domain heavy chain antibody fragment (nanobody) that specifically recognizes the surface of the protozoan pathogen *Trypanosoma brucei*. Nanoparticles were loaded with pentamidine, the first-line drug for *T. b. gambiense* acute infection. An *in vitro* effectiveness assay showed a 7-fold decrease in the half-inhibitory concentration (IC_{50}) of the formulation relative to free drug. Furthermore, *in vivo* therapy using a murine model of African trypanosomiasis demonstrated that the formulation cured all infected mice at a 10-fold lower dose than the minimal full curative dose of free pentamidine and 60% of mice at a 100-fold lower dose. This nanocarrier has been designed with components approved for use in humans and loaded with a drug that is currently in use to treat the disease. Moreover, this flexible nanobody-based system can be adapted to load any compound, opening a range of new potential therapies with application to other diseases.

1. Introduction

African trypanosomiasis is a disease with a devastating socio-economic impact in sub-Saharan Africa. The causative agent, *Trypanosoma brucei ssp*, is transmitted by the bite of and infected fly of the genus *Glossina* to humans or domestic livesock [1–3]. Human African trypanosomiasis (HAT), also known as sleeping sickness, is endemic in 36 African countries and around 60 million people are at risk of being infected. The prevalence has been variable during the twentieth century coinciding the re-emergence of the number of cases with periods of famine and war [4,5]. The public health situation has improved recently with surveillance and control efforts averting more than 1, 6 millions disability-adjusted life years in 2004. However, displacement of populations, conflicts, and poverty may lead to increased transmission, with severe social and economic consequences.

African trypanosomes are extracellular parasites transmitted by the bite of tsetse flies. Trypanosomes are able to evade the host immune system by changing the Variant Surface Glycoprotein (VSG), in a process termed antigenic variation [6,7]. The VSG is organized as densely packed dimers and works as a physical barrier impeding antibody recognition of invariant inner epitopes (Fig. 1). The rate of parasite surface turnover is very high and occurs in the flagellar pocket, which is an invagination of the plasma membrane around the base of the flagellum and the sole site for endocytosis [8]. This is a defence mechanism to degrade host antibodies bound to the surface of the parasite [9,10] but is also important for the uptake of some essential nutrients from the host blood, such as transferrin and lipoproteins [11,12].

Antigenic variation eliminates the possibility of developing an effective vaccine, leaving chemotherapy as the only method to fight against HAT disease. However, the drugs currently in use to treat

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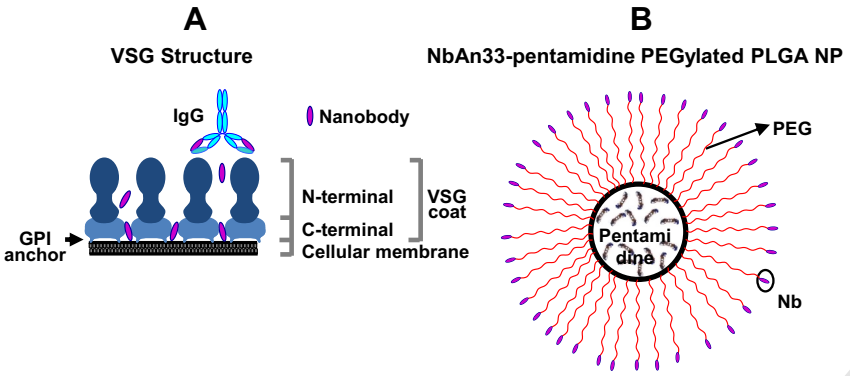

Fig. 1. Scheme of a conventional IgG antibody (A), a camelid heavy-chain IgG antibody (B) and the variable domain of heavy chain antibodies, VHH, also known as a nanobody (C). Conventional antibodies have two identical heavy chains, formed by V_H, C_H1, C_H2 and C_H3 domains and two identical light chains, V_L and C_L domains. Heavy-chain antibodies contain a single V_{HH} and two constant domains (C_H2 and C_H3). A Nanobody is the V_{HH} domain of heavy-chain antibodies obtained by recombinant gene technology. C_H1-3: constant domains of heavy chain. C_L: constant domain of light chain. V_H: variable domain of heavy chain. V_L: variable domain of light chain.

HAT are old and very limited, with most having serious side effects, including encephalopathy, toxicity and death [13,14]. Currently, there are only five licensed drugs for the treatment of HAT. Pentamidine and suramin are suitable for treating the disease before parasites invade the central nervous system; pentamidine is the drug of choice in the treatment of first-stage *T. b. gambiense* HAT, and suramin is used for first-stage *T. b. rhodesiense* HAT [13,14]. Eflornithine, nifurtimox or melarsoprol is the available drug for advanced disease. Clearly, alternative approaches in therapy are needed.

Nowadays, there are two approaches to develop new therapies: one is the search for new drugs and the other is the optimisation of actual formulations and their applications [15]. Design of nanoscale devices for drug-delivery is one of the most important goals in medicine and pharmaceutical technology [16]. Different systems based on nanoparticles (NPs) have been developed, including inorganic, magnetic and polymeric NPs [17]. These systems have many advantages when compared with conventional therapies. They protect drugs against oxidoreduction and enzymatic reactions, increasing the bioavailability and reducing the effective doses and negative side effects [17]. Incorporation of poly ethylene glycol (PEG) molecules on their surface (PEGylation) increases nanoparticle circulation times by reducing liver uptake. Nanoparticle PEGylation also provides moieties to attach biofunctional molecules for specific cell or organ targeting, such as antibodies [18,19].

Poly (lactic-co-glycolic acid) (PLGA) is a versatile polymer that is widely used for drug encapsulation through the formulation of NPs. PLGA is biodegradable and biocompatible and its use in humans for parenteral administration has been approved by the Food and Drug Administration and the European Medicine Agency [20]. The formulations and methods of PLGA NPs synthesis are well standardized and the NPs are adaptable to different types of drugs and administration routes. Moreover, their biological interaction with the environment and the rate of drug release can be modulated by modifying their physicochemical properties like shape, surface charge and hydrophobicity [21–23].

Nanobodies are single-domain antibody fragments derived from functional heavy-chain antibodies (HCAbs) of camelids [24–27] (Fig. 2). Unlike conventional antibodies, which are constituted of two identical heavy-chains and two identical light-chains, heavy-chain antibodies from camelids have lost the light-chains (Fig. 2). The heavy chain of HCAbs is composed of three instead of four globular domains: two constant domains (C_H2-C_H3) with high homology to conventional antibodies and one variable domain [28,29]. The C_H1 domain of conventional antibodies is lost. The variable domain (nanobody), can be cloned and expressed through recombinant gene technology, and is fully functional [30].

Nanobodies have several advantageous properties compared to conventional antibodies: i) small size (15 kDa), which is suitable for targeting epitopes in obstructed locations; ii) high affinity and specificity; iii) high stability and solubility; iv) not immunogenic to animals or humans; and v) well produced in bacteria and yeasts at low manufacturing costs [31]. Nanobodies have been successfully used for specific targeting in different therapeutic approaches which comprise direct blocking of receptors or viruses [32,33], chimeric fusion proteins [34–36], and conjugation to drug carriers [31,37–39]. Nanobodies have proven to be efficient for the treatment of infectious protozoan diseases, such as African trypanosomiasis [34] and malaria [40].

In the current study, we have taken advantage of the highly active endocytosis process to specifically and effectively deliver drugs into this protozoan parasite. The aim of this work was to develop a new polyvalent drug delivery system for the treatment of African trypanosomiasis based on PLGA NPs conjugated with a nanobody that specifically recognizes conserved cryptic epitopes on the parasite surface.

2. Materials and methods

2.1. Chemicals

Water used in the experiments was deionized and filtered with a Milli-Q Academic System (Millipore, Saint Quentin-en-Yvelines, France). All chemicals used were of analytical quality from Panreac (Barcelona, Spain), except for PLGA 50:50 [molecular weight (M_w): 12000 Da; inherent viscosity: 0.24 dL/g], dextran-70, polyvinyl

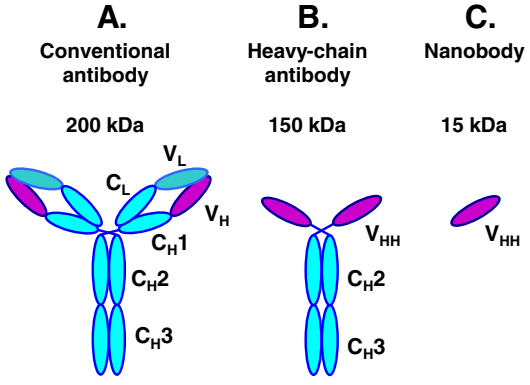

Fig. 2. A) Illustration of the trypanosome surface. NbAn33 recognizes a hidden epitope within the glycophosphatidylinositol (GPI) anchor of the variant surface glycoprotein. B) Illustration of a NbAn33-pentamidine-PLGA NP.

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