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Drug delivery systems in the treatment of African trypanosomiasis infections

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Introduction: Animal African trypanosomiasis (AT) is treated and controlled with homidium, isometamidium and diminazene, whereas human AT is treated with suramin, pentamidine, melarsoprol and eflornithine (DFMO), or a combination of DFMO and Nifurtimox. Monotherapy can present serious side effects, for example, melarsoprol, the more frequently used drug that is effective for both hemolymphatic and meningoencephalic stages of the disease, is so toxic that it kills 5% of treated patients. These treatments are poorly efficient, have a narrow safety index and drug resistance is a growing concern. No new drug has been developed since the discovery of DFMO in the 1970s. There is a pressing need for an effective, safe drug for both stages of the disease, and recent research is focused on the development of new formulations in order to improve their therapeutic index.

Areas covered: This review shows the potential interest of using nanoparticulate formulations of trypanocidal drug to improve parasite targeting, efficacy and, potentially, safety while being cost-effective.

Expert opinion: The design of drug formulations relevant to the treatment of AT must include a combination of very specific properties. In summary, the drug delivery system must be compatible with the physicochemical properties of the drug (charge, lipophilicity and molecular mass) in order to allow high drug payloads while being biocompatible for the patient.

Keywords: African trypanosomiasis, liposomes, nanoparticles, sustained release device, trypanocidal drug

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1. Introduction

African trypanosomiasis (AT) is a parasitic disease causing devastating epidemics in animal and human populations. The last one occurred in the 1970s. Thus, this severe tropical infection represents a public health problem [1,2]. In 1975, the World Health Organization (WHO) classified them into neglected diseases in order to attract attention to the need to develop more effective treatments to eradicate them and to avoid a re-emergence.

African trypanosomiasis affects 37 countries in Sub-Saharan Africa. One cause of the re-emergence in the 1970s was the lack of development of a medical arsenal available to veterinarians and physicians [3]. In point of fact, the main drugs registered to treat AT have mostly been used for > 50 years. They are classified as melaminophenyl arsenicals, diamidines, phenanthridines, polysulfonated naphturea and ornithines [3,4]. Their use presents severe hurdles, such as limited therapeutic efficacy, serious side effects and the emergence of resistant parasite strains [5]. Scientists are trying to develop new therapeutic molecules such as flexinidazole [6], or new combination chemotherapy, eflornithine (DFMO), alongside Nifurtimox, also named NECT [7].

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Owing to a lack of new drugs, scientists are turning to new galenic strategies such as drug delivery systems (DDSs) [8]. This approach seeks to improve drug therapeutic efficacy by designing a new delivery device containing the active molecule. This model is guided by optimization of the drug's pharmacokinetic properties and a reduction of its toxicity.

As compared with other therapeutic agents such as anticancer treatments, relatively few reports have been published on the delivery systems of antiparasitic agents. In this paper, DDS technologies are reviewed and their potential advantages for the treatment of AT are highlighted.

2. African trypanosomiasis

Pathogenic agents of AT are flagellated protozoa of the order Kinetoplastida and the genus *Trypanosoma*. They can infect humans and animals. The first identification of these parasites occurred in 1901, in Gambia, by the British scientists Forde and Dutton. Animal ATs are caused by *T. brucei brucei* (also named *nagana* in cattle), *T. congolense*, *T. vivax* and *T. evansi*. The causative agents of human AT, or sleeping sickness, are *T. brucei gambiense*, which is the causal agent of the chronic form in central and western Africa (90% of reported cases), and *T. brucei rhodesiense*, the causal agent of the acute form in east and southern Africa [2].

Trypanosomes are mainly transmitted to the mammalian host by a blood-sucking fly (genus: *Glossina*), which represents the vector responsible for the geographical spread of the disease. Trypanosomes present a polymorphism during their life cycle (**Figure 1**). The transmission of metacyclic trypomastigote parasite occurs from the glossine salivary glands to mucocutaneous tissue of the new host. In an early stage called the hemolymphatic stage, the parasite adopts a slender form and resides extracellularly in the bloodstream and the lymph nodes, then in organs. Trypanosomes are characterized by replication by means of fission and their ability to escape the host immune system. The strategy used is to mute the major variable surface glycoprotein (VSG), which is highly immunogenic [9]. The pathogenicity of a trypanosome involves a range of toxins that induce inflammatory reactions, tissue anoxia, anemia, heart failure and immunosuppression. In the meningoencephalitic stage, parasites cross the blood–brain barrier (BBB) and invade the cerebrospinal fluid (CSF). However, in the central nervous system (CNS) pathogens are protected from drugs [10,11]. The host progresses finally to death [5].

3. Chemotherapies

As trypanosomes are able randomly to modify their VSG coats, the development of an effective vaccine is too complex and chemotherapy (**Table 1**) remains the best option. Animal ATs are treated with homidium chloride, isometamidium chloride and diminazene aceturate, which all have therapeutic properties [12,13]: isometamidium and homidium also have a prophylactic activity. The first stage of human AT is cured with pentamidine isethionate and/or suramin sodium, and the second stage with melarsoprol or DFMO [2,3,13-14]. Nifurtimox (**Table 2**), marketed since 1960 for treating American trypanosomiasis, can also be used to treat AT in the two phases, but not in monotherapy. Recently, the WHO approved the use of a combination of DFMO and Nifurtimox (NECT).

Application of these antiparasitic agents is very delicate. They are often administered intravenously to ensure a rapid clinical response. This includes a series of daily injections with treatment duration depending on the host's parasitemia level [14]. Moreover, slow infusion is required for the administration of drugs with short drug half-life, poor solubility in water and narrow therapeutic index. Meanwhile, the necessity of laboratory and hospital equipment and the mobilization of medical personnel are often difficult to manage and too expensive in countries where sleeping sickness is endemic.

Moreover, in the cerebrospinal stage of human AT, the entry of drugs into the CSF and CNS is restricted by the structure of the capillaries and pericapillary glial cells that characterize the BBB. Diamidines, owing to the polar character of the active molecule, do not cross the BBB, which is not the case with DFMO, melarsoprol and nifurtimox. Nevertheless, these drugs induce neurotoxicity, especially melarsoprol (5 – 10% of fatal encephalopathy) [15,16].

Finally, uptake of melaminophenyl arsenicals and diamidines into the parasite's body requires carrier-mediated transport, probably via aminopurine transporters TbAT1 already existing in the parasite body [5,17]. However, if a parasite strain does not express this TbAT1 transporter it can be less sensitive to these treatments. This phenomenon is widely recognized and makes it even more important to conduct research into new vehicles for the delivery of trypanocidal molecules. The available chemotherapy treatments are therefore limited because they require long courses of parenteral administration, have variable efficacy and may demonstrate severe side effects (**Tables 1** and **2**). In addition, there is a real imbalance between production price and the expected therapeutic outcomes. Nevertheless, although these treatments are unsatisfactory, the fact that AT is a fatal disease justifies their use.

4. Expert opinion on DDS strategies

The drawbacks of trypanocidal chemotherapies justify the use of a DDS strategy whose ultimate goal is to optimize the drug absorption, distribution, metabolism and elimination (ADME). Moreover, galenic engineering efforts appear potentially more cost-effective and of shorter duration than the development of a new antiparasitic molecule.

The development of a DDS is used to improve the therapeutic effects of existing active molecules. The new formulation or device modifies the ADME properties with respect to the convenience and compliance of the patient. Obviously, this technology is applicable to a wide range of drug

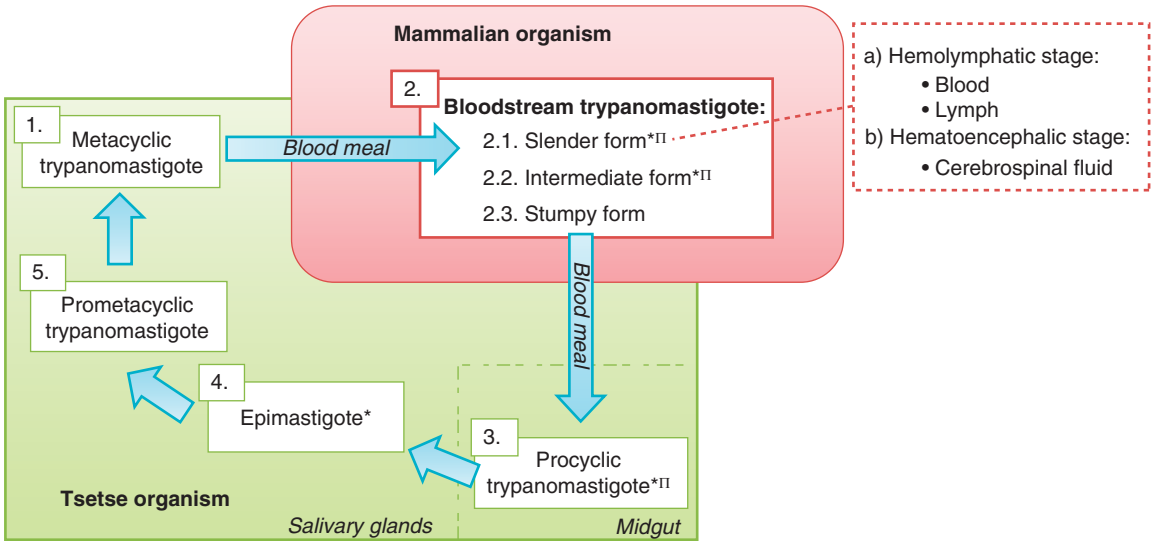

Figure 1. Life cycle of *Trypanosoma brucei* parasites. The *Trypanosoma brucei* parasite is transmitted to the mammalian host organism during the blood meal of the glossine. In fact, the saliva containing the metacyclic trypomastigote (1) is injected into the muco-cutaneous tissue of the mammalian organism. To enter into the blood and the lymph, the parasite adopts a slender form (2.1), which promotes its mobility. After the hemolymphatic stage, the parasite crosses the blood–brain boundary and reaches the CNS; this is the hematoencephalitic stage. To ensure its propagation, it takes a stumpy form (2.3) in the glossine blood, favorable to transport. In the midgut of the glossine, the procyclic trypomastigotes (3) are characterized by a replication via fission (*) and variable antigen coat (∏), which allows it to avoid being digested. It reaches the salivary glands as an epimastigote form (4), which can still replicate but loses this ability (5) and becomes a metacyclic trypomastigote (1), the form transmitted to mammals.

Table 1. Trypanocidal drugs for the treatment of animal African trypanosomiasis.

Pharmaceutical class ***Drug name***	**Pharmaceutical characteristics**	**Drawbacks**
Diamidine *Diminazene** (BERENIL®, VERIBEN®), 1955	Intramuscular and intravenous Uptake by carrier-mediated transport [91,92] Inhibition of the kinetoplasmatic DNA biosynthesis [93,94]	Licensed just for veterinary use Do not cross the BBB [95] Rapid decrease of the drug plasmatic concentration and slow elimination Side effects Numerous resistant strains [92,96-97]
Melaminophenyl arsenical *Melarsomine* (CYMELARSAN®), 1985	Intravenous, intramuscular Especially effective on domestic animals infected by *T. evansi* [98] and *T. equiderdum* – Not effective against *T. congolense* and *T. vivax* Oxidative stress	Resistant strains Rapidly metabolized in the plasma Side effects
Phenanthridine *Homidium*®‡ (*ETHIDIUM*®), 1952 *Isometamidium*‡ (SAMORIN®/VERIDIUM®), 1960	Intravenous, intramuscular Uptake by passive diffusion Inhibition of the DNA biosynthesis	Resistant strains Numerous side effects
Antimalinic *Quinapyramine*‡, 1949	Prophylactic treatment Used only in camels, horses and donkey Intravenous, intramuscular	Expensive use Numerous side effects Resistant strains [99,100]

*Group I compounds: produce their *in vivo* trypanocidal effect immediately.
‡Group II compounds: produce their *in vivo* trypanocidal effect only after a latent period of 24 h [101].

Table 2. Trypanocidal drugs for the treatment of human African trypanosomiasis.

Pharmaceutical class *Drug name*	Pharmaceutical characteristics	Drawbacks
Diamidine *Pentamidine*,* 1940	Therapeutic properties Intravenous, intramuscular $t_{1/2}$: 6 h after one injection and 12 days and more after a chronic treatment Uptake by carrier-mediated transport [91] Inhibition of the DNA biosynthesis [93,94]	Only effective against a *T. brucei gambiense* infection Does not cross the BBB [95] Rapid decrease of the drug plasmatic concentration and slow elimination Side effects Numerous resistant strains [96,97]
Melaminophenyl arsenical *Melarsoprol*®, 1949	Trivalent arsenical Intravenous [102] $t_{1/2}$: 35 h Narrow therapeutic index Uptake by carrier-mediated transport [91] Oxidative stress (inhibition of trypanothione reductase)	Poorly water soluble Rapid decrease of the plasmatic concentration and slow elimination Numerous side effects Treatment failure [14] and resistant strains Expensive treatment
Nitrofurane derivate *Nifurtimox,* 1977	*Per os* Mechanism not well known: oxidative stress (trypanothione reductase inhibition [103])	Salvage therapy in case of treatment failure with melarsoprol Serious side effects [104]
Polysulfonated naphturea *Suramin*®‡, 1920s	Polyanionic drug Intravenous, deep intramuscular Broad-spectrum anti-protozoal activity [105-107]	Does not cross the BBB [5] Slow elimination Expensive treatment Side effects Resistant strains [99]
Amino acid ornithine analogous *Eflornithine*®, 1981	Intravenous, *per os* $t_{1/2}$: 3.4 h because of high urinary clearance Specific and irreversible inhibition of ornithine decarboxylase [108]	Only effective against a *T. brucei gambiense* infection Large doses required for a patient (100 mg/kg) Cost of production is expensive Low side effects

*Group I compounds: produce their *in vivo* trypanocidal effect immediately.

‡Group II compounds: produce their *in vivo* trypanocidal effect only after a latent period of 24 h [101].

molecules, such as peptides, proteins, vaccines, genes and low-molecular-mass drugs (< 500 Da).

The formulation of a drug involves a variety of manufacturing methods and must consider multiple factors, such as solubility of drug molecules, administering an optimal dosage over optimal lengths of time, and protection of the pharmaceutical compounds from enzymatic degradation. At present, DDS research includes studies of sustained release formulations in order to minimize the number of doses necessary and targeted delivery systems, which ensure crossing of biological barriers and, sometimes, drug delivery near or in the diseased tissues or cells.

Therefore, DDSs are characterized by their preparation method, their composition, their size, their shape, and their inner and surface physicochemical properties. The main properties under consideration are the drug loading efficiency, dispersion stability, release kinetics, and *in vitro* and *in vivo* pharmaceutical behaviors.

To improve trypanocidal drug ADME, several DDS strategies are under development, such as formulation in liposomes, polymeric micelles, cyclodextrins and nanoparticles (NPs).

4.1 Liposomes

Liposomes are characterized by an aqueous reservoir volume delimited by one or several lipid bilayers. This system can entrap both hydrophilic and hydrophobic active molecules. They are classified by their size (20 nm to 10 µm) and the number of lipid bilayers that comprise them: small unilamellar vesicles (SUV); multilamellar vesicles (MLV) and large unilamellar vesicles (LUV). The first commercial pharmaceutical product using liposomes was Ambisome® (NeXstar Pharmaceuticals, San Dimas, California), a formulation of Amphotericine B. It was approved in 1997 by the US Food and Drug Administration to treat visceral leishmaniasis. Trypanocidal therapy potential of a drug encapsulated in liposomes has been reported for Trypanomastidae. The intravenous administration of liposomes passively targets organs of the reticuloendothelial system (RES): liver, spleen and lymph nodes [18]. Thus, Ambisome facilitates the targeting of pathogen agents (*Leishmania donovani*) characterized by intracellular localization in host macrophages [19]. Such targeting improves the drug efficacy and severely limits adverse effects such as nephrotoxicity on non-infected tissues. However, the

extracellular disseminated localization of AT trypanosomes makes the use of particulate system quite difficult.

4.1.1 Cytotoxicity of liposomes

Liposomes are able to present toxicity to different cell types depending on their component nature and ionic charges [20,21]. Liposomes composed either of stearylamine (SA):egg phosphatidyl choline:cholesterol (Chl) or dipalmitoyl phosphatidyl choline (DPPC):Chl present an *in vitro* cytotoxicity against *T. brucei gambiense*, *T. brucei rhodesiense*, *T. brucei brucei* and *T. congolense* [19,22-24]. The authors observed a deformation of the trypanosome body and an inhibitory effect against the motility of their bloodstream form. The trypanocidal power of SA-based liposomes may be explained by an interaction between their positive charge and the external surface of the parasite body, which is negatively charged, causing disturbance of membrane integrity and eventually cell osmolitic lysis [25]. The cell–vesicle fusion could offer the possibility of inducing specific modifications of the trypanosome plasma membrane, which would lead to its impairment [26].

Stearylamine and egg phosphatidyl choline were tested *in vitro* individually, but there was no trypanocidal effect, therefore the vesicular structure must be important. Furthermore, the *in vitro* toxicity observed using SA liposomes might be due to their observed toxicity in human cells, therefore limiting their potential use [18].

DPPC liposomes were tested *in vitro* on procyclic and bloodstream trypomastigote. Higher *in vitro* cytotoxicity was observed in bloodstream trypanosomes [22]. Balb/c mice infected by *T. congolense* were also treated by DPPC liposome at 20 µg/animal and presented a low efficacy improvement with 20% survival. These preliminary results suggest that liposomes should be used as carriers of trypanocidal drugs to treat AT. However, in order to have optimal biodistribution, liposomes should be stealth liposomes to avoid the mononuclear phagocytic system (MPS).

4.1.2 Trypanocidal drug encapsulated in liposomes

Cationic liposomes are recognized as powerful tools for DNA transfection into cells through fusion with cell membranes. In agreement with this idea, formulation of diminazene aceturate in SA-bearing liposomes was studied in a mouse model of *T. brucei brucei* and *T. evansi* infection [25,27]. This experiment highlighted that the encapsulated diminazene was more active than the free drug. Moreover, the diminazene liposomes showed slow absorption, few side effects, slow elimination and long duration of efficacy. Following *in vitro* tests, the drug penetrates thanks to a fusion between the liposomal membrane and the parasite plasma membrane [26]. This action can also induce cell lysis [24], suggesting a synergic effect between the loaded drug and the liposome carrier.

After intraperitoneal administration, however, liposomes are not stable and their biodistribution is not optimal to reach trypanosomes localized in blood, lymph and brain. Liposome aggregation and complement system activation might be responsible for their poor efficacy. This activation of the MPS occurs following adsorption of complement proteins (opsonins) [28]. To prevent this stage, one can modify the surface properties of liposomes by adding a hydrophilic steric barrier [29], lowering the interfacial energy [30,31]. This corona makes the carrier 'stealth' and thereby increases its blood residence time in the host organism [32].

4.1.3 Arsonoliposomes

Arsonolipids (1,2-diacyloxypropyl-3-arsonic acids) are lipidic analogues of phospholipids in which an arsenic group replaces a phosphate group in their polar head group [33]. The ability of pentavalent arsonolipid (As v) to be reduced to trivalent arsonolipid (As iii) by thiols (R-SH) is the major interest of these compounds [34]. Indeed, their potential oxidative power on thiol-containing enzymes has prompted the development of arsonoliposomes (As-lips), liposomes containing arsonolipids in their lipid bilayers. These DDSs were reported to demonstrate increased toxicity towards cancer cells and increased antiparasitic activity.

The trypanocidal activity of As-lips containing a palmitic acid acyl chain (C16) was evaluated by Antimisiaris *et al.* [35] and by Zagana and co-workers [36,37]. Three types of liposome were tested: C16-arsonolipid/egg PC/Chl; C16-arsonolipid/distearyl phosphatidyl choline (DSPC)/Chl; and C16-arsonolipid/DSPC/Chl + 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-PEG_{2000}. They were characterized in term of size, surface charge and membrane rigidity (saturated phospholipids forming more rigid membranes than unsaturated) [38]. PEGylation of the liposomes' coating leads to an increase of the diameter from 80 to 100 nm and a significant decrease of zeta potential from -25 to -3 mV [39].

In vivo tests were realized with the PC-based As-lips on an acute Balb/c mice model of *T. brucei brucei* infection with a single intraperitoneal injection of drug [37]. All mice were cured with PC-based As-lips without toxic effect [36]. However, the free drug (potassium melarsonyl) was effective at a lower dose. DSPC-based As-lips, PEGylated or not, were tested on a chronic Balb/c mouse model of *T. brucei brucei* infection. Both formulations showed no effect even at a dose five times higher than the effective dose of free drug, suggesting that they were not able to cross the BBB. The biodistribution of the three As-lip types showed that DSPC-based As-lip and PEG-As-lip loss after 5 min injection was ~ 30 – 40% of the injected dose, and no arsenic was detected in brain [37]. These studies thus suggest that, despite their membrane rigidity and improved biodistribution, they were not good candidates for treating the meningoencephalitic phase of AT because, whatever their composition, arsonoliposomes do not have the ability to cross the BBB.

4.1.4 Miltefosine liposomes

Miltefosine, or hexadecylphosphocholine (HePC), is part of the ether lipid class that is known for its apoptotic power on several tumor cell lines [40,41]. Miltefosine's mechanism of

action on trypanosome is not yet clear, but could include inhibition of phospholipids' and sterols' biosynthesis, as described in studies on Leishmania [42-45]. Miltefosine liposome was registered in 2002 in India as an oral treatment against visceral leishmaniasis. However, this treatment is expensive, and severe side effects such as gastrointestinal tract toxicity and teratogenicity were observed.

As for the arsonoliposome strategy, Papagiannaros *et al.* [46] developed an alternative approach in the trypanocidal treatment based on miltefosine liposomal formulation. It was a LUV composed of HePC/PC/SA (10:10:0.1) presenting a size of 104 nm and a zeta potential of -4 mV [46]. They observed that the use of miltefosine liposome enhanced by a factor of two the *in vitro* trypanocidal activity against *T. brucei brucei* and decreased slightly the *in vivo* toxicity of HePC. The authors suggested that their size was too large to be compatible with endocytosis uptake by the flagellar pocket of trypanosomes. The low increase in efficacy observed limits its potential use for further development.

4.2 Polymeric micelles

The term 'micelle' was first introduced by James William McBain in 1913 to characterize colloidal aggregation of low-molecular-mass surfactant in water at a concentration up to its critical micellar concentration (CMC). Nowadays, micelles also contain the self-assembly of either amphiphilic or oppositely charged polymers in aqueous medium. The hydrophilic and hydrophobic segments form, respectively, the corona and the core of the colloidal structure, with a size between 10 and 100 nm. Their properties are essentially dependent on the compound's nature, mostly the polar groups and the molecular chain length. Their advantages might be an improvement of the drug solubility and stability, prolonged circulation time and a controlled release for optimal targeting.

Diminazene was easily formulated in poly(amino acid)-based micelles resulting from electrostatic interactions between their carboxylic function ($pK_a \sim 4$) and the amidine functions of the drug molecule ($pK_a = 11$) in a Tris–HCl buffer at pH 5.3 [47-50]. The critical association concentration (CAC) value of diminazene/poly(amino acid)-based micelles depends on the chemical composition of the ionic diblock copolymer and on the level of drug loading within the micelle. One of the polymers commonly used to form polyion complex (PIC) micelles on interaction with an oppositely charged drug is poly(aspartate) (PAsp), owing to its biodegradability, biocompatibility and structural versatility. For PAsp to self-assemble into amphiphilic micelles, the poly(amino acid) segment must be either electrostatically neutral or conjugated to hydrophobic moieties. The copolymer PEG-PAsp formed micelles only in the presence of diminazene to the appropriate drug-polymer charge ratio [50]. In this preparation, the micellization was permitted as diminazene constituted the hydrophobic core and PEG the hydrophilic shell. Transmission electron microscopy studies confirmed that PEG-PAsp_diminazene complexes are discrete and fairly uniform in size and shape. However, the nanocarrier presented rapid drug release when the diminazene loading was too high. To improve this diblock system, Prompruk *et al.* added a third block, strengthening the lipophilic core with phenylalanine units (Phen): PEG-*block*-P(Asp-*stat*-Phen) [51]. The micellar size with the drug incorporation decreased from 150 to 50 nm with an increase of the amount of drug (diminazene:monomer 0.006:1 to 8.4:1 wt:wt). At lower pH values, the reduced ionization of the carboxylic acid groups reduces repulsive forces, leading to a dominance of attractive forces between the non-ionized portions and Phen moieties.

Another diblock copolymer used to formulate diminazene is carboxymethyldextran-*b*-PEG (CMDx-PEG), which can be designed as a substrate of tunable charge density, able to form polyion complex [49]. The micelles formed had a size of 36 – 50 nm and a unimodal size distribution (polydispersity index (PDI) < 0.1).

Diminazene was also used as a cationic drug model of micelles based on poly(ethylene oxide)-*b*-poly(L-glutamate) (PEO-PGlu) [48] and hydroxyethylcellulose-*g*-PAsp [52]. These systems were sensitive to some factors such as pH, concentration, temperature. Besides the dependence on ionic strength, the diminazene release behavior also depends greatly on the property of the carrier.

Moreover, the drug loading from PIC improves the entrapment efficiency and facilitates drug release from the nanocarrier. So far, no *in vitro* or *in vivo* studies on trypanosoma have been published using these PIC micelles.

4.3 Cyclodextrins

Cyclodextrins comprises six to eight glucopyranoside units bonded by α1–4 linkages constituting a cyclic oligosaccharide under a cone shape with a hydrophilic outer surface and a relatively hydrophobic internal space. This 'cage' is able to contain a guest lipophilic molecule.

The β-cyclodextrin (β-CDx) contains seven units and presents an internal diameter of 0.68 nm (α-CDx: 0.57 nm) [53]. It is often used to form inclusion complexes with poorly soluble compounds to improve their biological activity. The use of native β-CDx is unsatisfactory because it was found to be nephrotoxic by parenteral administration. To overcome this problem, a substituent can be added onto a glucopyranose unit such as randomly methylated-β-CDx (rame-β-CDx), dimethyl-β-CDx (dM-β-CDx), hydroxypropyl-β-CDx (HP-β-CDx) and sulfobutyl ether-β-CDx.

Gibaud *et al.* investigated the complexation of melarsoprol, the very toxic organoarsenical drug with either rame-β-CDx or HP-β-CDx to improve solubility, tolerability and bioavailability of this water-insoluble drug (log P = 2.53) [53]. They showed that both derivatives formed inclusion complexes at 1:1 stoichiometry ratio, which increased melarsoprol solubility 7.2×10^3-fold. *In vitro* experiments on a model of human cancer cell lines demonstrated that cytotoxic efficacy of the drug is not modified but rame-β-CDx slowed the melarsoprol

action. This phenomenon may suggest that the inclusion slows hydrolysis steps producing melarsenoxide, the active metabolite.

Ben Zirar *et al.* reported that the melarsoprol_HP-β-CDx formulation acted on the bioavailability and tissue distribution and should thereby improve the therapeutic efficacy of melarsoprol [54]. The inclusion complex following intravenous (i.v.) administration in mice promoted a higher concentration of melarsoprol in lipophilic tissues (i.e., brain and bone marrow). The new pharmacokinetics indicated that the acute toxicity was lowered compared with the free drug that induced cerebral toxicity. This is considered an asset in the treatment of the second phase of AT because only 3 – 5% of the administered dose of melarsoprol was detectable in the CSF [55].

4.4 Polymeric nanoparticles

Nanoparticle is a general name to describe nanocapsules and nanospheres [56]. According to the literature, a nanocapsule corresponds to a solid wall enveloping a liquid core (e.g., liposomes, lipid nanocapsules, etc.), whereas a nanosphere consists of a full or porous matrix (e.g., micelles, nanoparticles of maltodextrine, etc.). Polymeric nanoparticles represent solid nanostructures generally showing better stability than liposomes. They can be made from organic or inorganic materials, or a mixture of both. Organic nanoparticles are often made of either natural (collagen, gelatin, serum albumin, chitosan, maltodextrin) or synthetic biocompatible materials. The drug loading can be made by adsorption, entrapment or covalent attachment in the matrix, and binding can occur via hydrogen bonds, or electrostatic or hydrophobic bonds. In September 2008, the International Organization for Standardization (ISO) published Technical Specification ISO/TS 27687, 'Nanotechnologies – terminology and definitions for nanoobjects – nanoparticle, nanofibre and nanoplate' [57]. The nanoparticle specification refers to core terms such as the nanoscale (size range from ~ 1 to 100 nm); this includes liposomes and micelles. Nanoparticles are also defined as ultrafine (≤ 0.1 μm), while macroparticles are also called fine (between 0.1 and 2.5 μm) [58]. In fact, nanoparticles are complex systems owing to the presence of other components in the formulations, such as surfactants and drug; all these components modify nanoparticle behavior after injection in the body [28,59-60]. Nanoparticle suspensions have been developed as drug targeting delivery systems, using polyesters, poly(alkyl cyanoacrylate), poly(alkyl methacrylate-*co*-acrylic acid) and other polymers [61,62]. Drug release from synthetic polymer carriers may occur after polymer degradation, chemical cleavage of the drug from the polymer, swelling of the polymers and subsequent release of the drug entrapped within them, or osmotic pressure effects creating pores and releasing drugs by simple diffusion mechanisms. All these parameters show the great diversity and potential interest of such objects for nanomedicine applications. Moreover, it has been shown that NPs can cross the BBB *in vitro*; however, their *in vivo* transfer is still controversial [63-66]. These results suggest that these formulations may improve trypanosome therapy.

4.4.1 Nanoparticles of melarsoprol

Nanosuspensions of drugs are often prepared by high-pressure homogenization of the drug molecule and a small amount of surfactant is used as dispersant [55]. The size is affected by the product quality and the number of cycles. The use of specific surfactants avoids recognition by the RES and prolongs the blood circulation time. This technology is used for i.v. administration of poorly soluble drugs.

Ben Zirar *et al.* produced melarsoprol nanosuspensions with poloxamer 188 or 407 and mannitol [54]. The size was, respectively, 324 ± 88 and 407 ± 45 nm. Depending on melarsoprol concentrations, the size of nanosuspensions based on poloxamer 88 increased. They were stored after a freeze drying stage, which avoids the occurrence of aggregation phenomena. To limit the hydrolysis of melarsoprol and formation of melarsenoxide, the nanosuspension of melarsoprol must be administered immediately after reconstitution.

The tissue distribution in mice showed clear reticuloendothelial system targeting with five- to ninefold higher liver concentrations than the free drug injection; brain concentrations were three- to fivefold lower than the free drug. These results can be explained by their excessive size, which makes it impossible for them to cross the BBB. These formulations were not appropriate for testing on trypanosome-infected animals.

4.4.2 Nanoparticles of diminazene

For the formulation of hydrophilic drug by hydrophobic systems, the drug must be transformed to a lipophilic prodrug before the loading stage. The main strategy used is to graft a lipophilic group to the active molecule either by a covalent bond or an electrostatic bond to form a lipid drug conjugate (LDC). Olbrich and co-workers developed diminazene salts with distearate, tristearate and dioleate to form water-insoluble drug [67,68]. The diminazene_LDC formulations were performed by high-pressure homogenization of stearic acid and fatty acid drug, which form the matrix, and polysorbate 80 (Tween 80) as surfactant. The particle sizes varied between 250 and 450 nm and the surface charge was ~ -34 mV. The diminazene release from LDC particles is controlled by matrix degradation [69]. The emulsifier, Tween 80, was chosen for its potential ability to improve brain targeting following i.v. administration. Indeed, Kreuter *et al.* [63] highlighted that the organ distribution of particle DDS is strongly influenced by interactions with blood components, which is influenced by the surface properties of the carrier. Coating poly(butyl)cyanoacrylate NPs with Tween 80 increased their ability to deliver drugs to the brain. This surfactant has the ability to mediate brain delivery potentially by apolipoprotein (apo) E, apo-I and apo-IV adsorption, which may increase uptake via the LDL receptors at the BBB. This concept was called targeting by 'differential protein adsorption' [67].

The diminazene_LDC formulation was developed to deliver the drug to the brain by this strategy. The ratio of stearic acid: diminazene of the LDC formulations 2:1 and 3:1 influenced not only the diminazene loading efficiency, respectively, 33 and 25% (wt:wt), but also the amount of adsorbed plasma protein (apoE, apoA-I and apoA-IV). Moreover, Gessner *et al.* [69] demonstrated that the brain targeting was not due to adsorption of apoE but to apoA-I and A-IV, which might improve brain targeting of these NPs. They demonstrated *in vivo* that Nile red-labeled LDC particles adhered to the brain endothelial vessels and the marker was localized in the brain, as assessed by confocal microscopy [69].

Loiseau *et al.* [70] used cationic starch nanoparticles with a lipid core in which amphotericin B was loaded. They showed that after i.v. administration mice were cured of *Leishmania* infection. These nanoparticles were also shown to cross the BBB on an *in vitro* model [64]. Recently, Paillard *et al.* [71] showed that these nanoparticles did not induce complement activation. These results taken together suggest that these nanoparticles could be used for i.v. administration to target the brain. Furthermore, these NPs were loaded with diminazene and the authors showed that diminazene was stabilized from oxidative degradation and improved efficacy was observed on *T. brucei brucei in vitro* [72]. These NPs are good candidates for further studies.

4.4.3 *Nanoparticles of pentamidine*

Methacrylate polymers (PMAc) are well tolerated as cement for implants in humans for many years. PMAc NPs were prepared by emulsion polymerization, using a mixture of acrylic and methacrylic copolymers. Pentamidine methane sulfonate has been loaded in PMAc NPs by an ionic process involving the free carboxylic acid groups of polymer [73-75]. This formulation was tested in order to treat visceral leishmaniasis [74]. Indeed, Fusai *et al.* showed the injection of pentamidine loaded in PMac NPs in mice infected with *Leishmania major*, reduced significantly the blood parasite than the free drug [76,77]. The efficacy was six times better than free pentamidine, with good tolerability. *In vitro* tests showed that MAc NPs were taken up by the MPS, which could lower the interest in such NPs for treating AT. Moreover, MAc NPs were found to have a low biodegradability; so, pentamidine was formulated with polyester polymer as poly(DL-lactide), which is completely biodegradable and biocompatible [78]. This polyester is slowly hydrolyzed without enzyme action into lactic acid, a metabolite of the Krebs cycle [79]. This reaction is dependent on temperature and pH [80]. The poly(D,L-lactide) NPs were prepared by nanoprecipitation in the presence of Poloxamer 188 and lecithin. The pentamidine base binding percentage was influenced by the phospholipid concentration, which is limited by its solubility in acetone solvent. They obtained monodisperse colloidal suspension with a mean diameter between 131 and 154 nm and are stable for at least 9 months at 4°C. No *in vivo* studies on the AT animal model are herein reported.

4.4.4 *Bdellosome® of daunomycin*

Fricker and Flaig [81] patented in 2003 a solid NP system named Bdellosomes, whose size varies from 40 to 100 nm. This drug delivery system is made of polyvinyl alcohol grafted with polylactide side chains terminated by amino groups. The size and shape of the particle can be controlled by suitable modification of the polymer skeleton. These carriers can be loaded with substances of pharmacological interest, coated with PEG_{3400} both to evade immune reactions and to maintain their colloidal stability, and linked to target-specific ligands, such as transferrin or antitransferrin receptor-antibody fragments.

In vivo distribution studies showed prolonged circulation time of the PEGylated particles. They analyzed efficacy of daunomycin NPs against the bloodstream forms of *T. brucei*; the NPs were either plain or coated with PEG, transferrin, human serum albumin and transferrin antibodies [82]. Interestingly, trypanocidal activity was improved by transferrin targeting in the order: transferrin receptor antibody-coupled NPs > transferrin-coupled NPs > albumin-coated NPs > PEG-coated NPs > unmodified NPs.

4.5 Sustained release formulations

The use of biodegradable polymers provides a good opportunity to develop a sustained release system (SRD) that allows a slow release of the drug and prolongation of the prophylactic period.

4.5.1 *Sustained release devices of isometamidium and of homidium*

Biodegradable SRDs were prepared by extrusion of a mixture of poly(DL-lactide) (PLA) and isometamidium chloride or homidium bromide at 25% (drug:polymer wt:wt) under a cylindrical rod of 3 mm diameter and 3 cm long. Then they were coated with dexamethasone in order to reduce the tissue reaction at the implantation site in the animal's body.

The prophylactic activity of subcutaneously implanted homidium_SRD was assessed in rabbits, challenged with different stocks of *T. congolense* and compared with homidium [83-85]. The homidium_SRD formulation improved the rabbit's life from 30 to > 300 days, whereas no improvement was obtained with isometamidium_SRD. These formulations were also tested in cows. Two experiments were conducted, in which adult cows (520 – 799 kg) were subcutaneously implanted with SRD containing isometamidium or homidium and then exposed to tsetse flies infected with *T. congolense* at monthly intervals [86,87]. A significant extension of the prophylactic effect using the isometamidium and the homidium_SRD was noted by, respectively, a factor of 3.2 and 2.8.

To improve the PLA system, a copolymer of caprolactone (80%) and l-lactide (20%) was used. *In vitro* release studies using this polymer showed promising results [88,89]. This kind of copolymer has several advantages in a SRD for isometamidium: the protection period is extended as regards its

handling; and it is cheaper and easier to handle. However, the copolymer is less biodegradable than the homopolymer.

Lemmouchi and co-workers [87-90] developed polymer-drug formulations by extrusion using either poly(ε-caprolactone) (PCL) or PLA. They analyzed the drug release in phosphate buffer pH 7.4 at 37°C. They showed that the release of isometamidium is faster than for homidium. The first stage of homidium release was caused by diffusion, whereas for isometamidium a combination of diffusion and osmotic pressure effects played a role. The slow degradation of PCL explained the lower release of drug obtained [88].

4.5.2 *Sustained release devices of melarsoprol*

The development of melarsoprol_SRD is intended to avoid the injection of propylene glycol, which is the solvent of melarsoprol, and to improve its controlled release.

Microparticles of melarsoprol were formed with PCL either by the suspension-in-oil-in-water (S/O/W) solvent evaporation method (34 ± 17 µm) or by complexation of melarsoprol with methyl-β-cyclodextrin followed by the water-in-oil-in-water (W_{CD}/O/W) solvent evaporation method (31 ± 17 µm) [53]. The surface of the microparticles was observed by scanning electron microscopy: the S/O/W emulsion had a rougher aspect with crystals on the polymeric surface and the W_{CD}/O/W emulsion had a smooth aspect with potential cavities. The S/O/W microparticles presented a melarsoprol incorporation of 161 mg/g and an *in vitro* 50% drug release after 2 h and 80% after 7 h; whereas the microparticles W_{CD}/O/W containing only 2.89 mg/g of melarsoprol presented a faster drug release that was incomplete in their *in vitro* conditions (phosphate buffer pH 7.4, 30% propylene glycol, 37°C). The burst effect observed relied on breakage of the aqueous cavities embedded in the polymeric structure. Owing to the high drug release observed, no further studies were performed on AT-infected animals. In addition, as melarsoprol is extremely toxic it would be better to avoid its use, even with DDSs.

5. Expert opinion

The design of drug formulations relevant to the treatment of AT must include a combination of very specific properties. Indeed, the medical system must take into account the requirements due to the drug molecule, to its trypanocidal action and to patients. In summary, the DDS must be compatible with the physicochemical properties of the drug (charge, lipophilicity and molecular mass) in order to allow high drug payloads while being biocompatible for the patient. It must present a good stability during storage and in the bloodstream. It must be able to reduce the drug effective dose and its toxicity as compared with free drug by maintaining its therapeutic efficacy. Thus, the drug release kinetics that reflects its bioavailability and its pharmacokinetics must be optimized. By ensuring sustained release properties, it should reduce the frequency of drug intake. The use of a colloidal carrier must allow it to cross a series of biological barriers to deliver specifically the drug in trypanosomes nested extracellularly in the bloodstream, lymph nodes and CSF. Thus, the vector must present a size < 100 nm to improve its body distribution and be a stealth vector to avoid being captured by the macrophages of the RES and to optimize blood circulation time. To optimize further the targeting of trypanosomes, specific ligand molecules can be grafted on the vector surface. Chosen ligands must activate receptor on the luminal plasma membrane of endothelial cells in CNS vessels and potentially target also the parasite; this type of ligand is therefore not totally selective. To improve this system, the vector should have on its surface specific ligands to target the parasite and to allow brain delivery, such as transferrin. To increase the targeting selectivity, the immune carrier has to be a stealth carrier. Finally, the trypanocidal DDS should improve patient compliance by facilitating the drug administration route, should prolong the drug's effectiveness, and be cost-effective. Moreover, it should be able to act on drug-resistant strains. All these requirements make the development of DDSs to treat African trypanosomiasis difficult.

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