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Review

Drug delivery systems in HIV pharmacotherapy: What has been done and the challenges standing ahead

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ABSTRACT

Worldwide, over 40 million people are infected with the Human Immunodeficiency Virus (HIV). The High Activity Antiretroviral Therapy (HAART) combines at least three antiretroviral (ARV) drugs and, for over a decade, has been used to extend the lifespan of the HIV-infected patients. Chronic intake of HAART is mandatory to control HIV infection. The frequent administration of several drugs in relatively high doses is a main cause of patient incompliance and a hurdle toward the fulfillment of the pharmacotherapy. High adherence to HAART does not lead to complete HIV virus elimination from the host. Intracellular and anatomical viral reservoirs are responsible for the perpetuation of the infection. Active transport mechanisms involving proteins of the ATP-binding cassette superfamily prevent the penetration of ARV drugs into the brain and may account for the limited bioavailability after oral administration. A new research that addresses from simple organoleptic or technological problems to more complex issues involving the targeting of specific tissues and organs has emerged. With the aim to reduce dosing frequency, to improve the compliance of the existing pharmacotherapy and to target viral reservoirs, the design of drug delivery systems (DDS) is becoming complementary to new drug discovery. Based on the common molecular features that characterize the different families of ARV drugs, the present review describes state-of-the-art ARV DDS and thoroughly discusses the challenges in the development of medicines with enhanced biopharmaceutical properties. In addition, a number of specific issues such as pediatric HAART, preventive pharmacotherapy and specific HIV-associated ethical issues are addressed in an integrative manner. Finally, the impact of such novel drug development on the Pharmaceutical Technology field is discussed.

Contents

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1. Introduction

Worldwide, over 40 million people are infected with the Human Immunodeficiency Virus (HIV) [1]. The High Activity Antiretroviral Therapy (HAART) introduced in 1996 combines at least three antiretroviral (ARV) drugs [2–5] and, for over a decade, has been used to extend the lifespan of the HIV-infected patients (Table 1). In this context, the formerly fatal HIV-associated disease, acquired immunodeficiency syndrome (AIDS), has become a manageable chronic infection in most developed countries [6].

Chronic intake of HAART is mandatory to control HIV infection [7]; without it, viral replication resumes several weeks after withdrawal. Epidemiology reveals that optimal therapeutic results are attained when treatment adherence levels are greater than 95% (no more than two doses missed monthly in a twice-a-day regime); adherence levels below 95% could diminish therapeutic effectiveness by 50% [3,5]. The frequent administration of several drugs in relatively high doses is a main cause of patient incompliance and a hurdle toward the fulfillment of the pharmacotherapy [8]. Pediatric HAART is especially difficult. Only twelve ARV drugs have been approved for administration in children (as opposed to the twenty five approved for adults) [9,10]. Adult-approved ARV drugs are available only in solid form and have no corresponding pediatric forms [11], which is due to the fact that, until recently, companies in both the U.S. and the E.U. were not required to clinically test anti-HIV/AIDS medicines in children [12]. Liquid formulations are essential for pediatric pharmacotherapy; children under 7 are usually unable to swallow the solid medications [13]. To make adult medicines suitable for children, tablets or capsules are often processed to adjust dosages and facilitate swallowing [14]. As a result, ~40 million children are administered unlicensed medicines every year in Europe [15,16]. These extemporaneous formulations have risen significant safety, efficacy and quality concerns [17–19]. Organoleptic drawbacks that lead to avoidance and reduced adherence to ARV therapeutic regimes are often neglected [20]. Also, a decrease in the bioavailability of the drug has been found in many cases [21]. However, extemporaneous liquid formulations are the first choice to treat HIV infection in neonates and infants [22]. The last World Health Assembly recognized children's right to access safe, effective and proven medicines and approved the resolution "Best medicines for children" [23] and has recently launched the global campaign 'Make medicines child size' [23].

High adherence to HAART does not lead to complete HIV virus elimination from the host. Intracellular and anatomical viral reservoirs are responsible for the perpetuation of the infection [24–26]. Body compartments with blood–tissue barriers prevent drug penetration, thus preventing the eradication of latent viral pools. Active transport mechanisms involving proteins of the ATP-binding cassette (ABC) (e.g., P-glycoprotein, P-gp) that are present, for example, in central nervous system (CNS) prevent the penetration of ARV drugs into the brain [26,27]. Similar efflux pumps are found in the gastrointestinal tract and may account for the remarkable inter-individual variability of several orally-administered ARV drugs among patients [28,29].

A remarkable technological gap between the commercially available ARV formulations and the optimal features envisioned for a highly compliant ARV medication is apparent. In this context, a new research has focused on the design and development of ARV encapsulation and delivery strategies. These scientific works address issues ranging from simple organoleptic (e.g., unbearable taste) or technological problems (e.g., low aqueous solubility and stability under physiological conditions) to more complex issues involving the targeting of specific tissues and organs that serve as viral sanctuaries.

With the aim to reduce dosing frequency and to improve the compliance of the existing pharmacotherapy, the design of drug delivery systems (DDS) is becoming complementary to new drug discovery. The goal of the present review is to describe state-of-the-art ARV DDS and to thoroughly discuss the challenges in the development of medicines with enhanced biopharmaceutical properties. Several published reviews discussed different DDS designed to optimize the delivery of ARV drugs [30–32]. Contrary to this, the goal of the present article is to highlight the drawbacks that, based on to their common molecular features, characterize the different families of ARV drugs and the qualities that seriously hamper effective pharmacotherapy. Thus, a comprehensive overview of the different DDS approaches is presented for each ARV group. In addition, a number of specific issues such as pediatric HAART, preventive pharmacotherapy and specific HIV-associated ethical issues are addressed in an integrative manner. Finally, the impact of such novel drug development on the Pharmaceutical Technology field is discussed.

2. Drug delivery in antiretroviral therapy

Mirchandani et al. were early in stating a need for the development of ARV DDS to reduce the frequency of administration so as to improve the compliance and adherence to the treatment [33]. At the time of that pioneering work, only three ARV drugs were on the market and the treatment involved the administration of one ARV (monotherapy). Today, more therapeutic options are available [34] and, even if remarkably more effective, the pharmacotherapy has become more complex making the need for effective DDS more crucial [35]. The present section discusses the different approaches pursued to overcome the specific limitations shown by the different families of ARV drugs.

2.1. Nucleoside reverse transcriptase inhibitors (NRTIs)

NRTIs are prodrugs that require intracellular activation to become NRTI-triphosphates. Once activated, the drug competes with endogenous deoxynucleotide-triphosphate and inhibits the activity of the reverse transcriptase, a viral enzyme involved in the synthesis of the viral DNA from its RNA [36]. Zidovudine (AZT) was the first ARV developed and, upon approval in 1987, it became a key feature in anti-HIV therapy [37,38]. Main drawbacks of NRTIs are limited stability, first pass metabolism and systemic toxicity. For example, didanosine presents poor stability under gastric conditions (10% degrades within 2 min at pH<3 and 37 °C) and undergoes hepatic first pass. This results in low bioavailability. In addition, a twice-daily regimen is required during the first 6 months of HAART. In an early study, Abu-Izza et al. prepared AZT-loaded ethylcellulose microspheres by means of an emulsification/solvent evaporation method [39]. The variables affecting the properties of the DDS were the emulsifier concentration, the drug-to-polymer ratio and ethyl acetate concentration in the internal phase of the emulsion. The optimized microparticles released 85% in 6.5 h. Following a similar approach, Sanchez-Lafuente et al. produced matrix tablets from the water-insoluble and pH-independent polymers methacrylate and ethylcellulose by a direct compression technique [40]. Findings showed release profiles that adjusted to a Higuchi model and indicated a diffusive mechanism. Fine tuning of the polymer composition enabled

Table 1
Antiretroviral drugs and novel drug candidates under clinical evaluation.

Drug family	Drug	Commercial name (company)	FDA approval	Dosage forms	Adult dose	Pediatric dose
Nucleoside reverse transcriptase inhibitors (NRTIs)	Zidovudine (AZT)	Retrovir® (GlaxoSmithKline)	1987	Tablets (300 mg), capsules (100 mg), syrup (10 mg/mL).	600 mg/day (2 doses)	160 mg/m2 every 8 h
	Didanosine	Videx® (Bristol Myers Squibb)	1991	Capsules (125, 200, 250, 400 mg), oral solution (powder — 2 g), oral suspension (pediatric powder — 2 g), and tablets (25, 50, 100, 150 mg).	400 mg/day (1 dose)	120 mg/m2 twice daily
	Zalcitabine*	Hivid® (Hoffmann-La Roche)	1992	Tablets (0.375, 0.750 mg)	2.25 mg/day (3 doses)	Na
	Stavudine	Zerit® (Bristol Myers Squibb)	1994	Capsules (15, 20, 30, 40 mg) and oral solution (1 mg/mL)	80 mg/day (1 or 2 doses)	1 mg/kg/dose every 12 h
	Lamivudine	Epivir® (GlaxoSmithKline)	1995	Tablets (150, 300 mg) and oral solution (10 mg/mL)	300 mg/day (1 or 2 doses)	4 mg/kg twice daily (up to a maximum of 150 mg twice a day)
	Abacavir	Ziagen® ABC (GlaxoSmithKline)	1998	Tablets (300 mg) and oral solution (20 mg/mL)	600 mg/day (1 or 2 doses)	8 mg/kg twice daily (maximum of 300 mg twice daily)
	Emtricitabine	Emtriva® (Gilead Sciences)	2003	Capsules (200 mg) and oral solution (10 mg/mL)	Capsule: 200 mg/day Oral solution: 240 mg/day (1 dose)	6 mg/kg, maximum of 240 mg (24 mL) once daily
Nucleotide reverse transcriptase inhibitors (nRTIs)	Tenofovir	Viread® (Gilead Sciences)	2001	Tablets (300 mg)	300 mg/day (1 dose)	Na
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Nevirapine	Viramune® (Boehringer Ingelheim)	1996	Tablets (200 mg) and oral suspension (10 mg/mL)	First 14 days: 200 mg/day Alter 14 days: 400 mg/day (2 doses)	150 mg/m^2 once daily for 14 days followed by 150 mg/m^2 twice daily
	Delavirdine	Rescriptor® Sustiva.™, Stocrin.™ (Pfizer)	1997	Tablets (100 mg)	1200 mg/day (3 doses)	Na
	Efavirenz*	Sustiva® (Boehringer Ingelheim)	1998	Capsules (50, 100, 200 mg), tablets (300, 600 mg) and oral solution (30 mg/ml)	600 mg/day (1 dose)	10 kg<15 kg (200 mg), 15 kg<20 kg (250 mg), 20 kg<25 kg (300 mg), 25 kg<32.5 kg (350 mg), 32.5 kg<40 kg (400 mg), at least 40 kg (600 mg) once daily
	Etravirine	Intelence® (Tibotec, J&J)	2008	Tablets (100 mg)	400 mg/day (2 doses)	Na
Protease inhibitors (PIs)	Saquinavir	Invirase® (Hoffmann-La Roche)	1995	Capsules (200 mg) and tablets (500 mg).	2000 mg/day (2 doses)	Na
	Indinavir	Crixivan® (Merck & Co.)	1996	Capsules (100, 200, 300, 400 mg).	2400 mg/day (3 doses)	Na
	Ritonavir	Norvir® (Abbott Labs)	1996	Soft capsules (100 mg) and oral solution (600 mg/ 7.5 mL).	1200 mg/day (2 doses)	350 to 400 mg/m2 twice daily, not to exceed 600 mg twice daily
	Lopinavir	Aluviran® (Abbott Labs)	1997	Na	Na	Na
	Nelfinavir	Viracept® (Pfizer)	1997	Oral powder (50 mg/g) and tablets (250 and 625 mg)	2500 mg/day (2 or 3 doses)	45 to 55 mg/kg twice daily or 25 to 35 mg/kg three times daily
	Amprenavir	Agenerase® (GlaxoSmithKline)	1999	Capsules (50 mg) and oral solution (15 mg/mL)	2400 mg/day (2 doses — 24 capsules × dose)	20 mg/kg twice daily or 15 mg/kg 3 times daily (to a maximum daily dose of 2400 mg)
	Fosamprenavir	Lexiva® (GlaxoSmithKline)	2003	Tablets (700 mg) and oral suspension (50 mg/mL)	2800 mg/day (2 doses)	30 mg/kg twice daily, not to exceed the adult dose.
	Atazanavir	Reyataz® (Bristol Myers Squibb)	2003	Capsules (100, 150, 200, 300 mg)	400 mg/day (1 dose)	15 kg<20 kg 8.5 mg/kg once daily
	Tipranavir	Aptivus® (Boehringer Ingelheim)	2005	Capsules (250 mg) and oral solution (100 mg/mL)	1000 mg/day (2 doses)	14 mg/kg twice daily, maximum dose 500 mg.
	Darunavir	Prezista® (Tibotec, J&J)	2006	Tablets (75, 300, 400, 600 mg)	1200 mg/day (1 or 2 doses)	Na
Viral fusion/entry inhibitors	Enfuvirtide**	Fuzeon® (Hoffmann-La Roche)	2003	Powder (lyophilized) for injectable solution (90 mg/mL)	180 mg/day (2 doses)	6 to 16 years of age, 2 mg/kg twice daily. Maximum dose 90 mg twice daily
	Maraviroc***	Selzentry® (Pfizer)	2007	Tablets (150, 300 mg)	1200 mg/day (2 doses)	Na
	Vicriviroc***	Schering-Plough	Phase III	Tablets (30 mg)	30 mg/day	Na
Integrase inhibitors	Raltegravir	Isentress® or MK-0518 (Merck & Co.)	2007	Tablets (400 mg)	800 mg/day (2 doses)	Na
	Elvitegravir	Gilead Sciences	Phase III	Na	150 mg/day	Na
Maturation inhibitor	Bevirimat	Panacos Pharmaceuticals	Phase II	Tablets and oral solution (concentration Na)	600 mg/day	Na

Table 1 (*continued*)

Drug family	Drug	Commercial name (company)	FDA approval	Dosage forms	Adult dose	Pediatric dose
Fixed Dose Combinations (FDC)	Lamivudine/ zidovudine	Combivir® (GlaxoSmithKline)	1997	Tablets (150, 300 mg)	300 and 600 mg (2 doses)	****
	Abacavir/ lamivudine/ zidovudine	Trizivir® (GlaxoSmithKline)	2000	Tablets (150, 300 mg)	300 to 600 mg (2 doses)	****
	Lopinavir/ ritonavir	Kaletra® (Abbott Labs)	2000	Tablets (50, 200 mg), oral solution (80 mg/mL, 20 mg/ mL)	200 to 800 mg (1 or 2 doses)	7 kg<15 kg (12/3 mg/kg), 15 kg<40 kg (10/2.5 mg/ kg), <40 kg (400/100 mg) twice daily
	Tenofovir/ emtricitabine	Truvada® (Gilead Sciences)	2004	Tablets (200, 300 mg)	200 to 300 mg (1 dose)	****

*Available in a named-patient program only in US.
**Fusion inhibitor. Binds gp41.
***Entry inhibitor. Binds the CCR5 co-receptor.
****A fixed-dose tablet cannot be dose adjusted for the pediatric population.
Na: not available.

the modulation of the delivery profile; a gradual decrease in the dissolution rate of the drug was observed as the percentage of the more hydrophobic component (ethylcellulose) increased. This behavior stemmed from the plastic deformation of the polymer upon compression and the coating of drug particles. Deshmuckh et al. developed enteric-coated hydrogel-forming bioadhesive tablets to deliver small doses of the didanosine in a localized manner by means of bioadhesion to the intestine mucosa [41]. Different bioadhesive rate-controlling excipients such as Polyox® WSRN-303, Carbopol® 974P-NF, and Methocel® K4M were investigated. Tablets containing 10% Polyox WSRN-303 and 30% Methocel K4M showed release extents of 93 and 90%, respectively, after 12 h fitting the delivery profile of the Higuchi equation. In contrast, Carbopol® hampered the release of the drug due to a high drug–polymer affinity, thus proving unsuitable for the DDS. The mechanisms that governed the released were mainly diffusion and swelling of the polymer. Enteric-coated matrices were stable under stomach-mimicking conditions (0.1N HCl) and prevented the delivery and degradation of the drug [42]. Then, a fast dissolution of the enteric coating was found at pH 7.4. Finally, Polyox WSRN-303-containing tablets presented higher intestinal permeation than the commercially available formulation in *ex vivo* live intestine assays. Aiming to prevent the first pass hepatic degradation of didanosine, Lalanne et al. synthesized 1,3-dipalmitoyl glyceride-based glycerolipidic prodrugs of didanosine and didanosine monophospahte with the intention of direct lymphatic delivery [43]. The prodrugs were preformulated in liposomes and the cytotoxicity evaluated in phytohemagglutinin-P-activated peripheral blood mononuclear cells. Both didanosine derivatives appeared cell compatible. Then, cells were infected with the HIV-1-LAI strain and the antiviral activity of the prodrugs was measured by quantifying reverse transcriptase activity in the supernatant. While the didanosine prodrug showed similar activity to the free drug, the didanosine monophosphate derivative was slightly less active, likely due to the ionized nature of the molecule decreasing the cellular uptake. In a later study, the didanosine prodrug was formulated in emulsions, mixed micelles and liposomes for oral administration [44]. Due to the molecular modification, the drug had poor water solubility. Better solubilization was attained by means of taurocholate micelles, though concentrations were not sufficient for an oral formulation. A similar behavior was found in different oils investigated to develop an emulsion. Due to the phospholipid-like molecular structure of the prodrug with two hydrophobic chains and one hydrophilic head, its incorporation into dipalmitoylphosphatidylcholine liposomes was evaluated. High loading extents between 83 and 93% were achieved. Finally, in order to protect the drug from degradation under gastric conditions, drug-loaded liposomes were freeze-dried using sucrose as cryo-protectant and incorporated to gastro-resistant capsules. Another chemical modification to make NRTIs more hydrophobic was recently reported by Jin et al. [45,46]. They prepared amphiphilic cholesteryl self-assembled drug delivery systems (SADDS) of AZT and didanosine using different linkers (e.g., succinyl, adipoyl and phosphoryl). The derivatives showed poor water solubility, though nanosized 20–200 nm vesicle suspensions produced by the injection method (using THF as the solvent) remained stable at physiological conditions for at least 1 week. Other lipid-based carriers include algosomes [47].

To optimize the drug bioavailability, minimize the pill burden and prevent taste-related avoidance, several research groups have explored the potential of the transdermal (TD) route for the delivery of NRTIs. In a pioneering work, Seki et al. investigated the *ex vivo* permeation of AZT from a 12 mg/mL solution in isopropyl myristate containing 20% N-methyl-2-pyrrolidone as a penetration enhancer through rat abdominal skin [48]. Plasma levels around 1 μM were found 1–2 h after administration. Kim and Chien investigated the effect of different vehicles and skin permeation enhancers on the skin absorption of AZT, didanosine and zalcitabine or a three-drug combination solubilized in ethanol/water or ethanol/trycaprylin using hairless rat and human cadaver skin models [49]. The permeation rate for each drug (or the combination of drugs) was similar for both solvent systems. Addition of 1% oleic acid to ethanol/trycaprylin solutions did not improve NTRI permeation. In contrast, the use of a 0.3% oleic acid concentration improved the permeation of the individual drugs in ethanol/water, though the enhancer was less effective in improving the skin permeation of the drug combination. Remarkably, permeation through human skin was 3–4 times lower than in hairless rat skin. They also studied the effect of the volume fraction of ethanol in binary mixtures with water and trycaprylin on the permeation process [50]. Findings indicated a gradual increase in the permeation as the concentration of ethanol increased up ~50–60%. Ethanol concentrations greater than 60% resulted in decreased absorption. 1.0% oleic acid and N-methyl-2-pyrrolidone did not increase the permeation in ethanol/trycaprylin solutions. The opposite was observed in ethanol/water systems with the maximal enhancing activity being around 0.3%. Panchagnula and co-workers investigated the effect of different solvents [51] and additives (e.g., menthol and oleic acid) [52,53] on the skin permeation process of AZT formulated in solvent-based systems (e.g., water, ethanol and propyleneglycol and their binary combinations) [54,56] and a hydroxypropyl methylcellulose gel [55]. *Ex vivo* (rat skin) and preclinical studies suggested that plasma concentrations ~0.3 μg/mL could be attained [55]. These absorption extents are compatible with the required therapeutic levels (based on a 50-cm^2 patch, flux 500 μg cm^{-2} h^{-1} across human skin). However, due to the higher permeability of rat skin as compared to human, further studies are demanded.

To overcome side effects of orally-administered AZT, ethanol-rich vesicles (ethosomes) [54] were evaluated for TD delivery [55]. An optimized formulation displayed a transdermal flux of 78.5 μg/cm^2/h across rat skin. A more limited flux was observed when the drug was solubilized in hydroalcoholic and aqueous solutions with values of 5.2

and 7.2 μg/cm^2/h, respectively. In addition, findings suggested that the vesicles affected the structure of the stratum corneum resulting in a permeability increase. Lamivudine-loaded ethosomes were employed to elucidate whether the absorption mechanism of the drug involved intracellular or intercellular delivery [56]. Permeation extents with the ethosomal formulation (24.8–68.4 μg/cm^2/h) were 8-, 12-, 15-, 5- and 25-fold higher than a 2% phospholipid solution in ethanol (9.1 μg/cm^2/h), an ethanolic solution (6.5 μg/cm^2/h), a 45% hydroalcoholic solution (5.0 μg/cm^2/h), a liposomal system (12.6 μg/cm^2/h) and an aqueous solution (2.8 μg/cm^2/h), respectively. Moreover, encapsulation of lamivudine led to a decrease in the cytotoxicity of the drug *in vitro*. Finally, a significantly higher intracellular uptake by MT-2 cells was observed with the drug-loaded vesicles (85.7%) as compared to the free drug in water (24.9%) (Fig. 1).

Gerber et al. described the *in vitro* TD permeation of zalcitabine, lamivudine and N-acyl lamivudine esters by means of a submicron emulsion formulation (Pheroid®) through female human abdominal skin [57]. Despite the efficient entrapment of the different derivatives, the formulation did not improve the drug flux, which was likely due to the sequestration of the drug by the delivery system.

2.2. Nucleotide reverse transcriptase inhibitors (nRTIs)

Tenofovir is the only FDA-approved nucleotide reverse transcriptase inhibitor. Since tenofovir is already monophosphorylated the drug skips the activation stage required by NRTI [58]. No research works dealing with the relatively low bioavailability of the drug (25–30%) have been reported up to date [58]. A topical HIV microbiocide is a vaginally or rectally applied drug intended to prevent sexual transmission of HIV [59]. The pronounced increase in the number of women becoming infected (more than 50% of new infections) stressed the greater biological vulnerability of this subpopulation and motivated the search for a preventive pharmacotherapy. Using a novel approach, the efficacy of a 1% tenofovir gel was investigated in Indian rhesus macaques as a protective pharmacotherapy against HIV transmitted through anal intercourse [60]. The study was comprised of four study groups: (i) treated with 1% tenofovir gel per rectum up to 2 h prior to virus infection; (ii) placebo gel; (iii) untreated; and (iv) treated tenofovir gel 2 h after virus challenge. Findings showed the significant protective activity of tenofovir topical administration up to 2 h prior to virus challenge in 8 out of 9 animals. Untreated and two of three placebo cases were infected as well as two of three animals receiving the treatment after infection. Also, the degree of protection (six animals) was intimately associated with the plasma concentration of the drug 15 min after rectal application of the gel. Moreover, phase I clinical trials that evaluate the drug plasma concentrations following the topical intravaginal administration of uninfected pregnant women

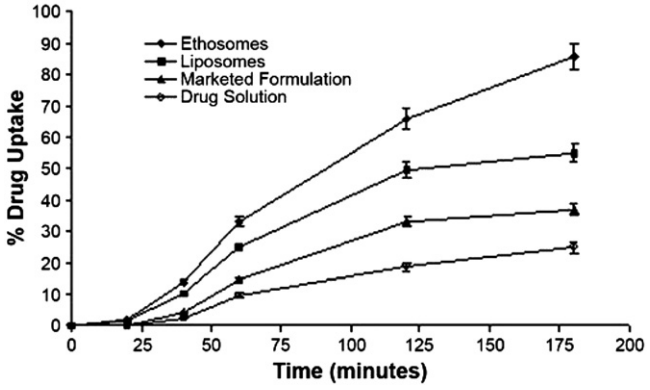

Fig. 1. Comparative cellular uptake of lamivudine after administering drug-loaded systems to MT2 cells. Values are mean ± S.D. ($n = 3$) (reproduced with kind permission of Springer Science+Business Media from ref. [56]).

who are expecting birth via caesarean section are being conducted [61]. This formulation "was chosen as a high priority microbicide candidate due to its activity in target cells for HIV infection of the vagina and cervix and the low frequency of local and systemic toxicity observed" [61].

2.3. Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

NNRTI drugs were first introduced in 1998 [62]. The mechanism of action involves the non-competitive binding of the drug to the reverse transcriptase enzyme. According to the guidelines, HAART usually includes at least one NNRTI as a first-choice drug [63–65]. Despite being used clinically for over a decade, the number of reports that deal with the technological aspects of these drugs is remarkably restricted. The British HIV Association, the US Department of Health and Human Services (DHSS) and the International AIDS Society (IAS) guidelines indicate efavirenz as the preferred NNRTI [64]. Efavirenz is also the NNRTI of election recommended by the WHO for the initial treatment of children above the age of 3 [66,67]. The very low solubility of efavirenz (~3–9 μg/mL) hinders its administration, absorption and biodistribution [68,69]; bioavailability is around 40–45%. In addition, it produces a burning sensation upon swallowing that precludes the development of water-based liquid formulations [70]. To make this drug more soluble and palatable, Bahal et al. solubilized the drug in a series of water-insoluble triglycerides [69]. This 30 mg/mL oily liquid formulation is available in a named-patient programme only in the U.S. where clinical studies are currently being conducted. Bioavailability levels are even lower than the solid form. Moreover, intake of large volumes of oily vehicles are expected to produce diarrhea. In other countries, no commercial pediatric formulations are available and preparation of extemporaneous suspensions appear as the only alternative in pediatric therapy. To improve drug solubility and dissolution, a recent study investigated the preparation of different efavirenz inclusion complexes with β-cyclodextrin (β-CD), hydroxypropyl β-CD (HPβCD), and randomly methylated β-CD (RMβCD) [71]. Three different preparation processes were used: (i) physical mixing; (ii) kneading; and (iii) freeze-drying. With β-CD, the apparent solubility of the drug increased linearly as a function of the CD concentration in the 0.002–0.3 M range, suggesting the formation of a complex with a 1:1 molar ratio. HPβCD and RMβCD showed a similar trend up to a CD concentration of 0.008 M. At higher concentrations, a 1:2 or higher molar ratios were observed. X-ray analysis showed that in the β-CD binary systems, the drug was crystalline. In contrast, HPβCD and RMβCD led to the formation of totally amorphous complexes, though a small amount of crystalline free drug was found in physical mixtures and kneaded samples. DSC experiments supported these findings. Dissolution assays were performed to evaluate dissolution kinetics and the total amount of drug dissolved. While physical mixtures showed a slight increase in solubility due to the higher wettability of the drug, a significant solubilization increase was apparent in the kneaded and freeze-dried samples. Finally, kneaded and freeze-dried HPβCD and RMβCD complexes showed the highest dissolution extents, being in the 10–20 and 6–8-fold ranges, respectively. Poor aqueous solubility is also a main drawback in the biological evaluation of new drug candidates. Yang et al. investigated a similar approach to solubilize UC-781, an experimental thiocarboxanilide NNRTI evaluated in the prevention of sexual HIV transmission [72]. UC-781 has shown extremely high anti-HIV-1 activity in cell culture, inactivating HIV virions and preventing cell-to-cell transmission. Moreover, the drug displays a "memory effect"; exposure of uninfected cells for periods of 15 min protected the cells from infection in the absence of exogenous drug. The poor aqueous solubility of the drug (30 ng/mL) is a hurdle that challenges the development of a topically-administered formulation. Complexation of the drug with 1.8 wt.% βCD, 40 wt.% HPβCD and 40% w/v MβCD increased the apparent solubility of the drug 20, 500 and 4000-fold, respectively.

According to the higher complexation constants determined for HPβCD and MβCD, the chemical modification of βCD appears to play a central role in the solubilization process. Methylation of βCD lengthens the hydrophobic cavity without distorting the geometry of the ring. Despite the usefulness of βCD, its limited solubility in water remains a challenge. The formation of inclusion complexes was confirmed by DSC where no endothermic peaks from melted drug were found. The anti-HIV activity of UC-781/ HPβCD containing drug concentrations of 1.5, 3 and 6 μg/mL was tested *in vitro* and compared to that of the free drug dispersed in an aqueous gel matrix (50 μg/mL). A dose dependent inhibition of the enzyme was apparent. The inhibitory activity (calculated as half maximal inhibitory potency) of all the complexes was substantially higher than that of the CD-free gel, stressing the relevance of the drug solubilization stage (Fig. 2).

Delivery systems for etravirine, an NNRTI drug that received approval in 2008, have yet to be developed, which is understandably due to the unknown pharmacokinetic parameters. However, due to its low solubility (10 μg/mL) and permeability, very low absorption extents are expected [73]. A recent etravirine study probed the bioavailability of pediatric (25 mg tablets) and adult (100 mg tablets) doses [74]. Thus, based on the available information, the need for improved pharmaceutical forms of this drug can be envisioned.

2.4. Protease inhibitors (PIs)

Protease inhibitor drugs are one of the pillars of the cocktail therapy. PI drugs are substrates for efflux pumps (see below) and so their oral absorption is restricted [75]. Because of their affinity for these removal transporters, the pharmacokinetic profiles depend on pharmacogenetic patterns and they require dose adjustment. This is a crucial issue in pediatric patients. Several PI drugs approved for adult therapies are still under clinical evaluation in children and they are not available in acceptable pediatric formulations; i.e., indinavir and the more recently approved atazanavir, tipranavir and darunavir [76]. In addition, it has been found that (i) the taste of some extemporaneous solutions (e.g., indinavir, tipranavir) is often unbearable for many children [77,78] and (ii) some liquids of acceptable palatability are less bioavailable than the original solid form [79]. For example, ritonavir, a PI incorporated into the HAART in low doses as boosting agent [80,81] is commercially available in pediatric aqueous solution. The extreme bitterness of this formulation hampers compliance [82,83]. In this context, Chiappetta et al. developed microparticles made of a pH-dependent pharmaceutical excipient in order to mask the bitter taste of indinavir sulfate [82]. The polymer matrix displays poor water solubility under intake conditions, minimizing the release of the drug. Once in a stomach-mimicking medium, the polymer quickly dissolves after protonation of the amine side moieties, thus releasing the drug. Palatability assays performed by healthy human volunteers supported the feasibility of the approach to render taste-masked 15.2 wt.% indinavir microparticles.

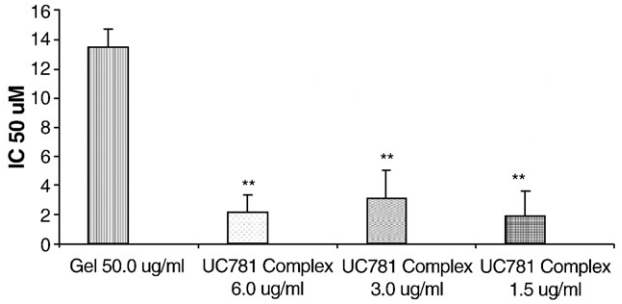

Fig. 2. Calculated IC_{50} for UC-781 in a CD-free formulation and in complexed systems. $^{**}P<0.01$ compared to the drug-containing gel (reproduced with kind permission of Springer Science+Business Media from ref. [72]).

The low aqueous solubility of saquinavir restricts absorption upon oral administration and biovailability is extremely low (4–10% depending on the formulation used). To overcome this limitation five 200 mg-capsules are administered twice daily. Aiming to improve the solubility of saquinavir in water, the complexation of the drug with different cyclodextrins has been pursued. Boudad et al. produced a hydroxypropyl-β-cyclodextrin–saquinavir inclusion complex [83]. The apparent solubility was increased up to 400-fold at pH 7; 10% CD led to solubility extents of 15.8 and 9.3 mg/ml at pH values of 7.0 and 2.0, respectively. The complex was incorporated into poly (alkylcyanoacrylate) nanoparticles produced by means of an emulsion polymerization; complexation significantly improved the entrapment efficiency of the drug during the production of the nanoparticles from 2.4–2.9 to 45–50 $\mu g_{drug}/mg_{polymer}$. To improve the solubility of the drug in water, Buchanan et al. extensively investigated the complexation of saquinavir free base and mesylate salt (a more soluble derivative, intrinsic solubility 2.1 mg/mL) with the new hydroxybutenyl-β-cyclodextrin [84]. Solubility levels increased to 6–12 and 3.8–12 mg/mL for the free base and the salt, respectively, with 10% CD. Then, an increase in the concentration of CD resulted in a gradual increase in solubility. Based on the molar ratios, multiple CD molecules interact with one saquinavir molecule with the ratio dependant on the drug form. Dissolution studies indicated that 95–100% complexed saquinavir dissolved within 30 min in a 1.2–6.8 pH range. In contrast, dissolution of uncomplexed saquinavir did not exceed 25%. Also, once dissolved, the CD prevented the crystallization of the drug in solution. Finally, pharmacokinetic assays were conducted. In general, a significant increase in the area-under-the-curve (AUC) from 2 to 18% was observed for the complexed free base drug as compared to the control. A similar approach was investigated to improve the absorption and bioavailability of nelfinavir. β-cyclodextrin complexes were produced using a milling technique [85]. When a 400 mg/kg dose was used in rabbits, a significant increase in bioavailability was apparent with the complex. At lower doses of the complex (200 mg/kg), the PK parameters were similar to that of a 400 mg/kg of uncomplexed drug.

To overcome low water solubility and intestinal absorption, poorly water soluble indinavir and saquinavir were also loaded into phosphatidylcholine and phosphatidylglycerol liposomes and the permeability studied *in vitro* in a Caco-2 monolayer [86]. Caco-2 monolayers are a model of intestinal epithelium enabling the measurement of the passive absorption and the energy-dependent efflux of drugs. Preliminary MTT and LDH assays carried out to evaluate cytotoxicity of the liposomes showed no significant detrimental effect on the mitochondrial metabolism and cellular membrane integrity. Permeation of both free drugs from water solutions in the apical-basolateral direction was very limited. The opposite was true in the basolateral-to-apical direction. Also, the addition of vinblastine, a known inhibitor of efflux pumps (e.g., P-gp), reversed this behavior and confirmed that both PI drugs are actively expelled from the cells. The drug concentrations attained by inclusion into liposomes was 10- to 2500-fold higher than those in water. Permeation experiments were conducted with 1/100 and 1/6.7 drug-to-lipid mass ratios for indinavir and saquinavir, respectively. Findings showed that liposomes diminished the apical-basal permeation of indinavir and saquinavir with respect to the aqueous solution, which likely stems from a lower concentration of drug available for passive permeation. On the other hand, the basal-to-apical active transport was pronouncedly inhibited, leading to an overall increase in the intracellular concentration of the drug. Aiming to improve the intracellular delivery of saquinavir to a THP-1 human monocyte/macrophage cell line, Shah and Amiji investigated the encapsulation of the drug within poly(ethylene oxide)-poly(caprolactone) (MPEG-PCL) nanoparticles prepared by means of a solvent displacement technique [87]. Findings showed significantly higher uptake extents with the drug-loaded nanocarrier as compared to the free drug

(Fig. 3). In a more recent work, a simple model to predict the intracellular concentration of the drug in cultured cells exposed to drug-loaded nanoparticles was described [88]. The processes considered in the model were (i) the drug release from the nanoparticles and (ii) drug and nanoparticle uptake by the cells. Both the relative kinetics of nanoparticle uptake and the drug release appeared as key players in governing the increase of the intracellular drug concentration. In another work, drug-containing oil-in-water nanoemulsions where the internal phase was composed of polyunsaturated fatty acids (oil droplet size 100–200 nm) were developed to increase the bioavailability of the drug in CNS, another anatomical reservoir [89]. *In vivo* assays showed higher plasma and brain drug concentrations following oral administration of the nano-formulations as compared to aqueous nano-suspensions. Bioavailability extents increased from 42.2% with the nano-suspension to 108.1 and 59.73% with flax-seed oil and safflower oil nanoemulsions, respectively. Moreover, the brain availability increased from 39.5% to 86.7 and 63.6%, likely due to brain's selective uptake of polyunsaturated fatty acids. Also, encapsulation resulted in a slight decrease of the plasma bioavailability from 100% for the nano-suspension to 71 and 89.8% for flax-seed and safflower oil nanoemulsions, after intravenous administration. In contrast, delivery to the brain was markedly increased by 364.52% and 141.97%, respectively. The higher effectiveness with formulations containing flax-seed oil as compared to safflower oil could be explained by the higher omega-3 fatty acid concentrations.

In a unique strategy, Dou and co-workers loaded indinavir nanoparticles into bone marrow-derived macrophages [90,91]. The drug-loaded systems were administered intravenously in mice and the drug levels monitored in the spleen, liver, lung, kidney, sera and urine. Sustained release of indinavir was observed in tissue and sera for up to 10 days. *In vivo* experiments in HIV-infected mice indicated a significant reduction in the number of infected cells in different tissues and organs following the administration and sustained antiretroviral activity. In addition, immune reconstitution was apparent after 14 days.

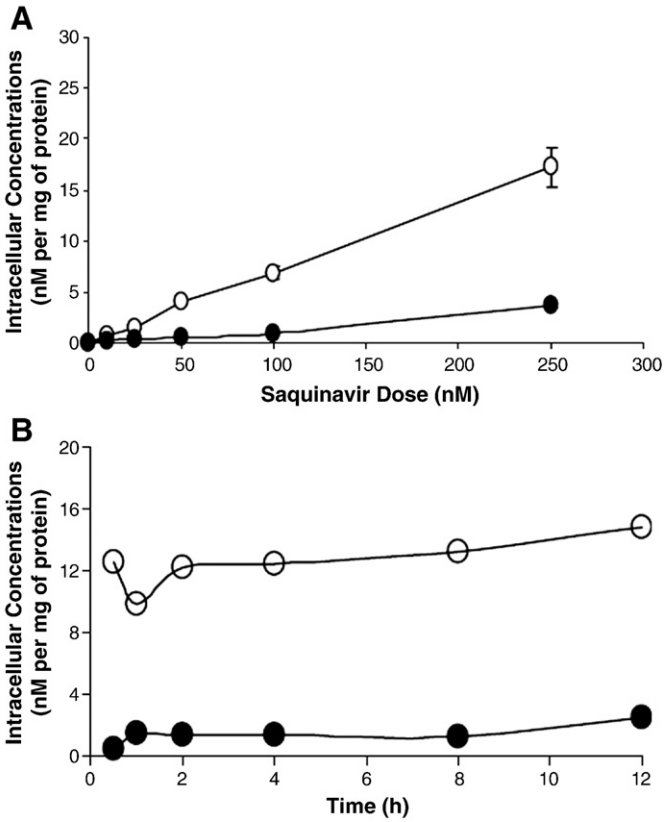

Fig. 3. Intracellular concentrations of saquinavir *versus* the administered dose (A) and incubation time with a constant drug concentration of 50 nM (B) to a THP-1 cell line. Tritiated [^{3}H]-saquinavir was administered in aqueous solution (filled circles) and encapsulated within MPEG-PCL nanoparticles (empty circles) (reproduced with kind permission of Springer Science+Business Media from ref. [87]).

2.5. Novel ARV drugs: a tantamount of novel drug delivery challenges

In order to improve the efficacy of treatment and to overcome viral resistance, new drugs are being designed. In 2008, etravirine, a new non-nucleoside reverse transcriptase inhibitor (NNRTI) for administration in patients with established resistance to other ARV drugs was approved by the FDA [92]. Additional pathways in the process of viral replication are also being targeted. Entry/fusion inhibitors are among the most recently introduced ARV; this multi-step process is an essential stage in the pathology of the infection [93,94]. Enfuvirtide, a peptide that mimics aminoacids 127–162 of HIV-1 gp41 and inhibits its fusion, was the first drug approved in this group in 2003. Due to its instability in the gastric environment, enfuvirtide is administered subcutaneously twice a day. Upon injection, local irritation and pain are observed. To overcome the adverse effects, a gas-powered needle-free drug delivery device (Biojector® 2000, Bioject Medical Technologies) has been clinically evaluated [95]. Despite encouraging preliminary findings that suggested significantly lower injection site reactions, pharmacokinetic bioequivalence and higher patient compliance, it was ultimately abandoned due to insufficient improvement [96]. In this framework, the development of a DDS protecting the drug from degradation and enabling efficient absorption upon oral administration appears as a challenging goal in the years to come. Integrase inhibitors are another new family of ARV [97]. Raltegravir was approved in 2007 for administration in treatment-experienced patients. Elvitegravir is undergoing phase III clinical trials [98]. The last candidate undergoing clinical evaluation is the maturation inhibitor bevirimat, which is still in phase II studies. Results demonstrated that upon oral administration of the drug, immature viruses were non-infectious [99,100]. These novel ARV consequently challenge the design of more convenient drug delivery systems. Due to their more recent approval, information is still incomplete (e.g., bioavailability) and a large dimension of the technological problems is unknown. Clinical experience over the coming years will probably identify the biopharmaceutical weaknesses of each agent and motivate further investigations to overcome the disadvantages.

3. Targeting of viral reservoirs

Regardless the remarkable progress made in ARV pharmacotherapy, HIV is able to conserve its replication machinery in anatomical and intracellular sites where the ARV drugs have restricted access. HAART does not eliminate these reservoirs, nor prevent their generation and hence, a rebound in viral plasma levels occurs upon HAART withdrawal [101]. CD4+ T lymphocytes are the best investigated reservoir [26,102]. Others reservoirs are the cells of the mononuclear phagocyte system (e.g., monocytes/macrophages, dendritic cells and Langerhans cells), the brain, hepatocytes and the gastrointestinal tract [103].

Numerous technological approaches aiming to improve the effectiveness of the treatment by targeting different cellular and anatomical viral reservoirs are being pursued. In this framework, the use of nanoparticles has arguably become the most attractive research avenue for targeting monocytes/macrophages [103] the CNS [104,105] and the gastrointestinal tract [106]. Nanocarriers display a number of advantageous features: (i) poorly water soluble or unstable drugs can be hosted within the particle to attain improved solubility and stability under physiological conditions; and (ii) they are up-taken by phagocytic cells. In early studies, Lobenberg and co-workers developed AZT-loaded hexylcyanoacrylate nanoparticles using bis(2-

ethylhexyl) sulfosuccinate sodium as a surfactant and evaluated their biodistribution upon peroral and intravenous administration in rats [107,108]. The oral administration resulted in higher plasma levels than the free drug in solution and a more effective delivery to the cells of the reticuloendothelial system (RES). Moreover, following IV injection, drug concentrations were found to be up to 18-fold higher in the cells of the mononuclear phagocyte system with the drug-loaded nanoparticles as opposed to the control solution [108]. Further studies showed that the AUC for the nanocarrier in the liver was 30% higher than the control. A similar trend was found in the blood and the brain. A number of indinavir-lipid complexes with disteroyl phosphatidylcholine and methyl polyethylene glycol-disteroyl phosphatidylethanolamine have been also investigated to enhance the delivery to lymphatic tissue upon subcutaneous administration [109,110]. In general, higher drug concentrations (up to 6-fold) in both peripheral and visceral lymph nodes and a more prolonged exposure in the blood were found.

Surface-unmodified nanocarriers remain relatively non-specific vectors, however further progress demands increased reservoir specificity. In this context, the present section discusses a number of rationally tailored surface modifications that have been explored over the years for cytosolic and nuclear delivery of ARV drugs. It is worth stressing that this research is at the academic level and none of these potentially effective strategies have reached the stage of clinical trials or FDA approval.

3.1. Targeting of the cells of the immune system

3.1.1. Immunoliposomes and other lipid-based carriers

Liposomes appeared as the first nanocarrier system proposed for intracellular HIV targeting. Pioneering work on the delivery of AZT from liposomes was conducted by Phillips and Tsoukas in the early nineties [111]. This DDS was also administered intravenously in a phase I study to infected patients with end-stage disease with acceptable results [112]. Pretzer et al. evaluated the antiretroviral activity of a poorly water soluble experimental PI, L-689,502, loaded into liposomes, on monocyte-derived macrophages infected with HIV-1 [113]. Results showed that encapsulation within multilamellar liposomes was more effective than the free drug, with the EC_{90} of the liposomal system being 2.9 and 4.5-fold lower. Aiming to enhance the selectivity of the delivery, liposomes bearing anti-HLA-DR antibodies were designed and evaluated by Bergeron et al. [114–118]; the HLA-DR ligand of the major histocompatibility complex II (MHC-II) is present in different lymphoid cells and tissues. Immunoliposomes preferably bonded to cells expressing high levels of the MHC-II *in vitro*. Also, they showed better accumulation in the lymphatic system upon a single subcutaneous injection. In contrast, other organs (e.g., liver) did not show especially higher levels when compared to the unmodified liposomes. Moreover, Stealth® immunoliposomes displayed better accumulation extents in all the tissues [118]. In a more recent work, the biodistribution of free indinavir and drug-loaded liposomes and immunoliposomes was evaluated after a single subcutaneous injection over the course of 2 weeks. Significantly higher indinavir concentrations were found in lymph nodes, liver, spleen and plasma [118]. Then, the *in vitro* antiviral efficacy of HIV-1-infected PM1 cells was assayed. Both liposomal and immunoliposomal indinavir were efficient as a free drug. However, immunoliposomes enable the targeting of HIV lymphatic reservoirs and thus appear as a promising alternative *in vivo*. Moreover, evaluation of the toxicity on hepatic activity following ten consecutive subcutaneous administrations to mice indicated no toxic effects. Aiming to overcome the relatively fast clearance of conventional liposomes *in vivo*, the incorporation of AZT into acetylated-low density lipoprotein (AcLDL) was explored [119]; AcLDL is taken up by macrophages via scavenger receptors. In order to improve the limited affinity of the hydrophilic drug for the lipophilic carrier, a 5′-*O*-13-oxamyristate-AZT prodrug was synthesized. Also, 13-oxamyristic acid has been reported as a strong inhibitor of the *N*-myristoylation of viral proteins and the cleavage of the prodrug molecule would release two antiviral molecules. Two techniques were studied for the incorporation of the prodrug: the contact and the microemulsion. The latter was found to be more effective; loading efficiency increased from 32 and 47% for the free drug to 61 and 281% for the prodrug. Incubation of prodrug-loaded systems in fresh human blood showed their high stability under *in vivo*-mimicking conditions. Two cell lines expressing scavenger receptors were used to evaluate cellular uptake: J774.A (mouse macrophage) and U937 (human monocyte). A 10-fold increase was apparent with the AcLDL carriers as compared to the free drug. Moreover, addition of a 30-fold excess of AcLDL to the culture medium inhibited the uptake, confirming the specificity of the mechanism.

3.1.2. Cell-penetrating peptides

Cell-penetrating peptides (CPP), also known as protein transduction domains (PTD), have been evaluated for the direct delivery of drug-loaded nanoparticles to the cytoplasm or even the nucleus of different cells in an energy-independent manner [120,121]. Although the translocation mechanisms are still unclear [122], it is likely that basic or cationic aminoacids like lysine and arginine, abundant in CPP, interact with the negatively-charged cellular membrane. Among the CPP, the trans-activating transcriptor (TAT) sequence present on the surface of the HIV-1 [123,124] and other sequences containing 8–9 arginine consecutive units (poly-arginine) [125] are the most studied as they have been investigated in various preclinical models [126]. Grafting of TAT peptides to the surface of different types of nanocarriers has been performed with the intention to guide the fate of particles for drug delivery and diagnostics. Torchilin et al. designed several types of TAT surface-grafted 200 nm-liposomes and evaluated the interaction with different tumor cell lines [127]. When the peptide was directly bounded to the surface of the nanocarrier or when long PEG chains (MW 5 kDa) were also attached to stabilize the liposomes, the steric hindrance prevented the TAT-liposome/cell interaction. In contrast, the interaction was not hindered when the TAT was linked via a PEG spacer or when the liposomes were modified with shorter PEG (MW 3 kDa) segments. Soon thereafter, the effective cellular delivery of a fluorescent model drug was observed. The internalization was also investigated in the presence of diverse metabolic inhibitors. Low temperature conditions (4 °C) did not affect the process suggesting that it is energy-independent. Similar results were obtained with sodium azide (inhibitor of oxidative respiration) and iodoacetamide (a blocker of the cytoskeleton assembly). Finally, analysis of the integrity and the intracellular delivery of the modified liposomes indicated that the nanocarriers remain intact after 1 h. In a more recent study, Fretz and co-workers investigated the uptake of TAT-liposomes by human ovarian carcinoma cells (OVCAR) *in vitro* under similar inhibitory conditions [128,129]. Contrary to the results reported by Torchilin, a clear inhibition of the internalization was found under low temperature conditions or in the presence of iodoacetamide and citocalasine (an anti-actin agent) and an endocytotic mechanism was proposed. Zaho et al. conjugated the TAT peptide to superparamagnetic iron oxide particles and evaluated their utility as non-invasive diagnostic means to track different cell types by MRI [130]. Findings indicated a concentration-dependent behavior (the higher the TAT concentration, the more efficient the cellular uptake) and sensitivity levels 100-fold higher than those currently described. Independently of the internalization mechanism, the use of TAT surface-modified nanoparticles appears as a promising approach for the ARV targeting of HIV sanctuaries in the immune system (see below in CNS).

3.1.3. Other peptides

A number of peptides that mediate an energy-dependent cell uptake have been investigated to target cells of the immune system. *N*-formyl peptides are products generated by the cleavage of bacterial

and mitochondrial proteins and display a potent chemo-attractant activity for mammalian phagocytic leukocytes [131]. Wan et al. covalently bound the most active synthetic macrophage chemo-attractant peptide sequence, N-formyl-Met-Leu-Phe (fMLF), to poly (ethyleneglycol) (PEG) nanocarriers and evaluated their macrophage uptake *in vitro* [132] and *in vivo* [133]. Preliminary studies indicated that the affinity of the nanocarrier for phagocytic cells increased as a function of the number of fMLF residues per PEG molecule [132]. In a later work that aimed to optimize the molecular features of the nanocarrier (e.g. MW and number of fMLF copies), the uptake by undifferentiated and differentiated (to macrophages) human U937 cells was studied [133]. The former expressed a low concentration of fMLF receptors (FPR and FPRL1). In contrast, the latter displayed high levels of receptors on the surface. PEG-fMLF nanocarriers bearing 1, 2, or 4 peptide copies significantly increased the uptake by differentiated cells between 1.5 and 3.8-fold [133]. The uptake was substantially reduced at 4 °C, indicating the active nature of the mechanism involved. To evaluate the influence of the PEG MW on the process, PEG-$(fMLF)_2$ molecules with 5, 20 and 40 kDa PEG precursors were investigated. An uptake improvement (14.4%) was observed with a PEG size increase from 5 to 20 Da. Then, a further increase in the size resulted in a pronounced decrease of 38.6%. According to these results, nanoparticles displaying hydrodynamic radii in the 20–60 nm range (20 kDa) appear as the most appropriate for targeting. *In vivo* experiments in mice showed that PEG5000-fMLF improved the peritoneal macrophage uptake by 3.8-fold compared to a PEG control after intraperitoneal injection. An increase in the fMLF/PEG molar ratio led to a gradual increase in the uptake; e.g., PEG5000-$fMLF_2$ and PEG5000-$fMLF_4$ resulted in ~11 and ~24-fold increases, respectively (Fig. 4). The accumulation was similar in macrophages of the liver, kidneys and spleen [133].

Dutta et al. modified poly(propyleneimine) (PPI) dendrimers with tuftsin, a natural macrophage activator tetrapeptide (Thr–Lys–Pro–Arg), for the encapsulation and delivery of efavirenz [134]. The peptide is physiologically released upon the enzymatic cleavage of IgG. The protection of the terminal amine group of PPI is a means to improve the cytocompatibility of these nanocarriers. Conjugation of tuftsin increased the drug loading from 0.65 to 0.87 $g_{drug}/g_{carrier}$ and entrapment efficiency from 37.4 to 49.3%. *In vitro* drug release studies showed a significantly slower release profile for tuftsin–PPI. The unmodified dendrimer released 100% of drug within 24 h. Contrary to this, the modified version extended the release substantially (79% in about 6 days). MTT assays indicated a significant decrease in the cytotoxicity of the tuftsin-dendrimer as compared to the pristine PPI. Cellular uptake studies of the free drug and drug-containing PPI and tuftsin–PPI by uninfected and infected Mo/Mac cells were conducted. In uninfected cells, efavirenz–PPI systems showed an initial significant increase compared to the free drug followed by a rapid loss in cellular viability 4 h later due to the cytotoxic effect of the dendrimer. Tuftsin–PPI resulted in 34.5 and 19-fold increases after 1 and 4 h, respectively. In addition, HIV-infected cells showed a significant increase in the uptake of modified PPI. Finally, the efavirenz encapsulation within tuftsin–PPI increased the anti-HIV activity by 42-fold compared to that of the free drug *in vitro*.

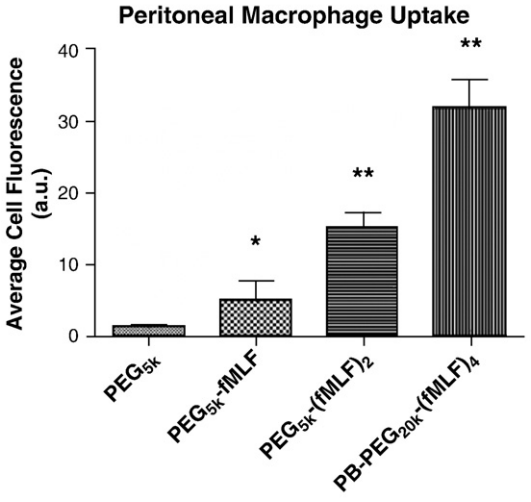

Fig. 4. Uptake of PEG-fMLF nanoparticles by mice peritoneal macrophages at 37 °C after 4 h of incubation. Means ± S.D. ($n = 3$). *$P<0.05$, **$P<0.01$ (reproduced with kind permission of Springer Science+Business Media from ref. [133]).

3.1.4. Lectin-recognizable sugars

Grafting of saccharides recognizable by lectin receptors located on the surface of phagocytic cells to the surface of different types of nanocarriers (e.g., liposomes, dendrimers and nanoparticles) has been pursued to improve the delivery of ARV drugs to lymph nodes. Jain et al. have extensively explored this approach to selectively deliver AZT [135] and stavudine [136,137] from drug-loaded liposomes and to minimize systemic exposure and manifestation of adverse effects. *In vitro* release assays of stavudine from galactosylated liposomes showed a zero-order profile and a slower release as compared to uncoated liposomes due to the steric hindrance of galactose residues on the surface. Hepatic uptake of the modified nanocarriers after IV injection was significantly higher than that of the free drug. Main toxic effects of stavudine are haematological (decrease in leukocyte, erythrocyte, Hb, platelet and polymorphonuclear count) and hepatic, the effects after a single day. Uncoated liposomes delayed the onset of toxicity to day 10, while no toxicity was observed with coated liposomes. In addition, a remarkable modification of the biodistribution was found; e.g., lesser renal elimination and higher uptake by liver, spleen and lungs. A similar modification was employed to target the delivery of efavirenz [138] and lamivudine [139] from polypropylene dendrimers. This modification has been also been used to modify solid nanocarriers. The NRTI drug didanosine was encapsulated in gelatin nanoparticles and the drug-loaded nanocarrier coated with mannan by means of an incubation technique [140]. *In vitro* release profiles indicated that encapsulation of the drug in uncoated and coated nanoparticles significantly retarded the release of the drug. An aqueous solution released 100% of the drug after 2 h while uncoated and mannan-coated systems delivered 48.8 and 42.5%, respectively, after 24 h. Phagocytosis experiments indicated a sharp increase in the cellular uptake from 21.7% for the free drug in solution to 51.2 and 88.7% for the unmodified and mannan-modified particles, respectively, after 2 h. This phenomenon led to an enhanced didanosine uptake from 12.1 to 37.2 and 62.5% for the uncoated and coated systems. Biodistribution studies following subcutaneous injection showed a higher accumulation of the nanoparticles in target organs such as the spleen, the lymph nodes and the brain and lower extents in the liver and the kidneys. Mannan-coated nanoparticles showed the best performance with a 12-fold increase in both the lymph nodes and the brain compared to the solution. Similar results were observed when the particles were coated with mannose molecules [141]. Overall, this approach has been shown to be a highly versatile alternative to modify a broad variety of nanocarriers displaying different structural features and chemistries.

3.2. Targeting of the CNS

The blood-brain-barrier (BBB) restricts the passage of a broad spectrum of hydrophilic and hydrophobic ARVs drugs to the CNS. In addition, the presence of efflux transporters that remove the absorbed drug in the basolateral-apical direction leads to the generation of one of the most challenging viral reservoirs [105]. For example, the P-glycoprotein (P-gp) is expressed in brain-microvascular endothelial cells and constrains the access of PI. Accumulation in the CNS not only

generates a virus pool that curtails the total elimination of the HIV from the host but also may lead to neuroinflammation, neurodegeneration and dementia (HIV-1 encephalitis, HIVE) [106]. As opposed to the more extensive investigative work performed on the targeting of immune cells by means of different surface modifications, selective delivery to the CNS has been the subject of more limited study. A number of works reported the enhanced delivery of ARV drugs to the brain by means of encapsulation into polymeric (e.g., polybutylcyanoacrylate) and lipidic nanoparticles [142,143]. In general, *in vitro* permeation assays are conducted using human brain-microvascular endothelial cells, an *in vitro* model of the blood-brain barrier (BBB). For example, Chattopadhyay et al. studied the potential of solid lipid nanoparticles (SLNs) for the enhanced delivery of atazanavir to the brain [144]. Findings showed higher accumulation extents in the endothelial cell monolayer, as compared to a free drug aqueous solution. However, since this strategy does not comprise any mechanism of selective recognition by cells of the BBB, extensive investigations are still required to determine the fate of the nanocarriers *in vivo*. Aiming to target the brain *in vivo*, Mishra et al. tested transferrin (Tf)-modified PEGylated albumin nanoparticles (sizes ~120 nm) containing AZT [145]; transferrin receptors are expressed on the surface of brain endothelial cells and mediate the endocytosis of transferrin. *In vitro* release studies showed that successive PEGylation and transferrin modifications slightly lowered the release extents from 41.7% for unmodified nanoparticles to 39.5 and 38.4% for PEG-NP and Tf-PEG-NP, after 6 h. To evaluate the targeting to the brain, FITC-dextran loaded NP were administered intravenously and the fluorescence in the brain observed with microscopy. PEG-NP and NP controls showed a weak and diffuse fluorescence. In contrast, a higher intensity was found with Tf-modified NP. Biodistribution of AZT-loaded NP showed that the free drug is quickly cleared from plasma, while all the NP prolonged the circulation times. Results of drug distribution *in vivo* showed a significant increase in the percentage of drug recovered from the brain for the Tf-PEG-NP (Fig. 5).

The modification of nanocarriers with CPP to target cells of the immune system has been described above. In a recently published work, ritonavir-containing TAT-coated PLA nanoparticles were produced and evaluated *in vitro* and *in vivo* [146]; the peptide was conjugated to the polyvinyl alcohol associated with the nanoparticles at the interface. *In vitro* transport studies with Madine Darby canine kidney cells over-expressing P-gp (MDCK-MDR1) and non-P-gp-expressing MDCK-wild type (MDCK-wt) cells showed a significant increase in permeability with the modified nanoparticles as compared to the free drug and the unmodified particles, with the phenomenon being more pronounced in MDCK-MDR1 cells. In the case of P-gp-expressing cells, the uptake was 0.04, 0.59 and 3.36 $\mu g_{drug}/mg_{protein}$ for the free drug and the unmodified and modified nanoparticles, respectively. In wild type cells, a more moderated increase from 0.29 for the free drug to 0.83 $\mu g_{drug}/mg_{protein}$ for the TAT-modified systems was found. Biodistribution experiments showed higher ritonavir concentration in the brain with the free drug 3 h after of an IV injection. However, at later time points, drug levels were significantly higher in animals receiving the encapsulated drug; e.g., 0.1, 80.3 and 12.2 $\mu g/g_{tissue}$ for free drug, TAT-conjugated and unconjugated nanoparticles, respectively. These findings represented an 800-fold increase with the modified carriers. Moreover, fluorescence analysis of coronal sections of the mouse brain indicated the accumulation of TAT-nanoparticles in the parenchyma, confirming the translocation of the carrier into the CNS. Finally, drug levels attained were even higher than the therapeutic concentrations previously indicated, supporting the potential of this strategy to eradicate the virus from the CNS.

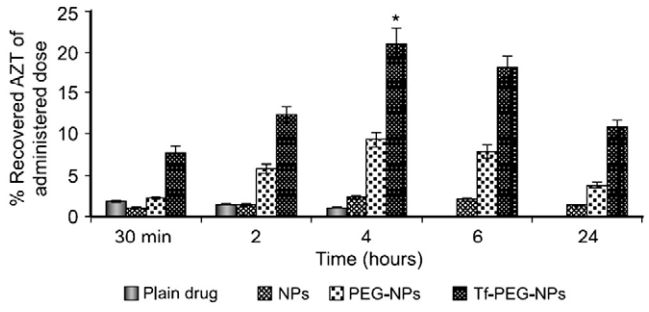

Fig. 5. *In vivo* localization of albumin (NP), PEGylated albumin (PEG-NP) and transferring-modified PEGylated albumin (Tf-PEG-NP) nanoparticles in the brain. Means ± S.D. ($n = 3$). *$P < 0.01$ (reproduced with kind permission of Informa Healthcare from ref. [145]).

3.3. Targeting of the liver

Molecular modification of nanocarriers to direct drug delivery to the liver has been sparsely investigated. Chimalakonda et al. conjugated lamivudine to dextran to selectively deliver the drug to the liver [147]. The accumulation of the conjugate in the liver was 50 times higher than the free drug. Even though this work was intended for the treatment of hepatitis B infection, this strategy appears as a promising one for the targeting of hepatic reservoirs.

4. Improving the drug penetration to viral reservoirs by the inhibition of efflux transporters

ATP-binding cassette (ABC) transporters like P-gp and the multidrug resistance-associated proteins (MRP) are well identified mechanisms that inhibit oral drug absorption [148,149] and penetration through the blood–tissue barriers delimiting virus reservoirs in the brain and testes. For example, PIs and NRTIs are main substrates of P-gp [150] and MRP [151], respectively. An illustration of the relevance of the efflux transport phenomenon is the fact that indinavir was initially categorized as a Class IV drug according to the Biopharmaceutics Classification System due to its apparently low permeability after oral intake [152]. However, later on, the drug was reclassified to Class II, indicating the ability of the molecule to intrinsically cross the intestinal barrier in the absence of active efflux mechanisms [153]. In addition, a study by Hochman et al. demonstrated that the P-gp efflux in the basolateral-to-apical direction increased the exposure of indinavir to a drug-metabolizing enzyme in the intestine (cytochrome P-450), resulting in increased degradation extents and lower bioavailability [75]. To overcome this drawback, PI drugs are usually administered in combination with a low dose of ritonavir, a PI that effectively inhibits both the activity of P-gp and the enzymatic degradation machinery. Very recently, the role of P-gp in limiting the access of the NRTI abacavir was also established [154].

Over the years, Kabanov et al. have extensively investigated the potential of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) block copolymers (PEO–PPO–PEO, Pluronic®) in the inhibition of efflux transporters. His work mainly focused on cancer resistance [155]. More recently, these polymers have been observed to improve the absorption and bioavailability of ARV drugs, ultimately becoming useful carriers in anti-HIV pharmacotherapy. Spitzenberger et al. showed that Pluronic® P85 increased the biodistribution a zidovudine/lamivudine/nelfinavir cocktail (ART) in the brain due to the inhibition of P-gp in blood–tissue barriers [156]. *In vitro* preliminary studies showed that drug-free 0.01–0.001% P85 reduced the viral replication in infected monocyte-derived macropages (MDM) by 55 and 44%, respectively. Also, an enhanced antiviral activity of nelfinavir was found when it was combined with 0.01% P85 *in vitro*. When administered to a severe immunodeficiency mouse model of HIVE, higher brain exposure to the ARV and a significantly lower infection level was apparent when the drug combination was supplemented with Pluronic® P85 as compared to the drugs alone (Fig. 6). For example, the initial 39.9 and 68.5% infected MDM cells were decreased to 9.4 and 7%, at days 7 and 14, respectively, with a 0.2% P85/cocktail treatment. In contrast, a polymer-

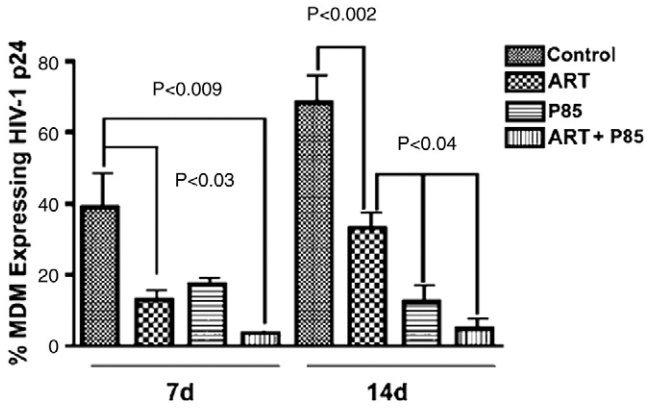

Fig. 6. Effect of P85 (0.2%) and triple drug combination on viral replication in the mice HIVE model. Means ± S.D (reproduced with kind permission of Nature Publisher Group from ref. [156]).

free ARV combination showed a more moderated effect with 35.5% reduction at day 14. Remarkably, a 0.2% drug-free P85 solution reduced the viral load to 13.4%, supporting the intrinsic antiviral activity of the amphiphile.

In more recent studies, the inhibitory activity of Pluronic® P85, F88 and F127 on the accumulation of nelfinavir and saquinavir in multidrug resistant cells (e.g., MDCKII and LLC-PK1 transfected with MRP1) was evaluated [157,158]. While P85 inhibited both the basal and the nelfinavir-induced P-gp activity, their more hydrophilic counterparts (F127 and F88) were not active. A similar trend was observed when the accumulation of the drug was evaluated. P85 also increased the accumulation of saquinavir with this effect being more pronounced in MDR cells. In addition, increasingly higher polymer concentrations above the critical micellar concentration (CMC) had a detrimental effect [157]. The mechanism involved is still unclear though sequestration of drug molecules by the increasing number of polymeric micelles could explain, at least partially, this phenomenon. This feature together with the ability of the copolymers to generate polymeric micelles [159] creates interesting opportunities in the design of unique drug delivery systems addressing both technological and biopharmaceutic challenges in HIV/AIDS pharmacotherapy.

5. A new ethical issue: intellectual property rights *versus* access to medication

A comprehensive overview of the different approaches for the design of ARV drug delivery systems has been introduced in the previous sections. It is noticeable that an important proportion of the developmental work in recent years takes advantage of different nanotechnologies. A late report by Cientifica Ltd. forecasted a dramatic growth nano-DDS market from the current $3.4B (about 10% of the global drug delivery market) to ~$220B in 2015 ($26B in 2012) [160]. As opposed to cancer, a disease that affects people in both developed and developing countries, HIV/AIDS has a greater impact on poorer societies. Emerging ethical issues can be defined at two levels. The first level is related to the access to conventional ARV formulations. Lawmakers in developing countries that are members of the Trade Related Aspects of Intellectual Property Rights (TRIPS) are forced to pass stricter patent laws. A representative example is the Indian patent law that went into effect on January 1st 2005 [161]. If implemented in its original form, it might have constrained the production of generic anti-HIV/AIDS medicines, risking patient access to this medication. Voices around the world advocated for a more flexible bill and currently a commitment from governments, international agencies and pharmaceutical companies has made prices of brand name medications more affordable, especially in low income countries, where agreements between multinational and local companies enable the synthesis of the Active Pharmaceutical Ingredient (API) and production of the final formulation at lower prices. The second level has to do with the severely limited access of HIV-infected patients to more sophisticated (and expectedly much more expensive) technologies. In this context, it is still unclear whether the design and development of ARV DDS will benefit broad portions of the infected population or, remain constrained to a smaller, more affluent group of patients.

6. Challenges for an already-here future

While a cure for HIV/AIDS remains a long-term commitment, our immediate and midterm challenges are to design cost-effective treatments and therapies with improved features that would: (i) reduce intake frequency; (ii) increase the bioavailability and decrease the degradation in the GI tract; (iii) improve the taste and stability of liquid formulations; (iv) inhibit the activity of efflux pumps in the different tissues and organs; and (v) target cellular and anatomical viral reservoirs. Realizing these challenges would transform a certainly fatal disease into a chronic one, regardless of patient socioeconomic status. Due to high costs, dosing difficulties, fewer pediatric-approved drugs, limited number of liquid formulations and difficult registration issues, the situation for pediatric patients is more dire. A recent review work delineates the immediate goals to improve the treatment of HIV-infected infants and children [162]. In this context, as a first step to accomplish high patient compliance and therapeutic success, DDS research is poised to become the tool to develop safe, stable, easy-to-use and, more importantly, economically affordable treatments. Finally, a geriatric patient subpopulation is emerging as a consequence of successful HAART pharmacotherapy; ~3 M infected patients over 50 years of age live with the infection. This phenomenon will necessarily open new research avenues. No matter the vicissitudes of pharmacological technologies, the future, with purposeful drug design intimately coupled with their mode of efficient delivery, should continue towards improved health and accessibility for all.

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