ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 44 (1997) 127–132

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

Body distribution of azidothymidine bound to nanoparticles after oral administration

R. Löbenberg, L. Araujo, J. Kreuter *

Institute of Pharmaceutical Technology, Frankfurt, Germany

Received 28 November 1996; accepted 10 April 1997

Abstract

Reticuloendothelial cells play an important role in the immunopathogenesis of AIDS. For this reason, a targeted delivery of antiviral drugs to these cells should significantly improve therapy of AIDS. The objective of the present study was to investigate the possibility of specific drug targeting of antiviral drugs to the reticuloendothelial cells by the oral route. Hexylcyanoacrylate nanoparticles were used as colloidal drug carriers for azidothymidine (AZT). ^{14}C-labelled AZT was bound to nanoparticles using bis(2-ethylhexyl) sulfosuccinate sodium as surfactant. The radioactivity in several organs including those containing large numbers of macrophages was measured after peroral administration of the nanoparticle preparation and compared to a [^{14}C]AZT control solution containing the same components without the nanoparticles. In the liver the area under the curve (AUC) of [^{14}C]AZT was 30% higher when the drug was bound to nanoparticles than after administration of the solution. In addition, higher [^{14}C]AZT concentrations were observed after 1 hour and at later time points in blood and brain when nanoparticles were used compared to the control solution. These results indicate that nanoparticles are a promising drug targeting system for nucleoside analogues. Furthermore, the increase in drug availability at sites containing abundant macrophages, e.g. in the blood and in the brain, may allow a reduction in dosage and a decrease in systemic toxicity. © 1997 Elsevier Science B.V.

Keywords: AZT; Body distribution; Drug targeting; Nanoparticles; Macrophages

1. Introduction

Reticuloendothelial cells act as a reservoir for the Human Immunodeficiency Virus (HIV) and are believed to be responsible for its dissemination throughout the body and into the brain [1,2]. To target anti-AIDS drugs to these cells, therefore, is a promising strategy in AIDS therapy [3].

After i.v. injection colloidal drug carriers such as nanoparticles have been shown to accumulate in the macrophages [4,5]. Orally administered nanoparticles were found to be taken up by the gut [6–9]. There are certain mechanisms suggested to be responsible for the peroral uptake [10], for example intracellular uptake described by Kreuter et al. [11] or a paracellular route reported by Damgé et al. [12]. A mixed uptake via both routes was reported for iron particles by McCullough et al. [13]. However, the major site for the uptake of colloidal drug carriers seems to be the M-cells of the peyer's patches [14]. Up to 60% of the uptake is accounted by these cells of the gut [10].

Azidothymidine (AZT) is still one of the most important drugs in AIDS-therapy [15], but side-effects like bone marrow toxicity or haematological changes minimize the ability for a consequent antiviral therapy. As shown in earlier in vitro studies with human macrophage tissue cultures, AZT bound to nanoparticles retains its full antiviral activity [16]. The in vitro

* Corresponding author. Institute of Pharamceutical Technology, Johan Wolfgang Goethe-University, D-60439 Frankfurt Main, Germany. Tel.: +49 69 79829682; fax: +49 69 79829694.

PII S0939-6411(97)00078-7

efficacy of other antiviral drugs, e.g. the poorly bioavailable protease inhibitor saquinavir [17] was enhanced 10-fold [18] when it was bound to nanoparticles.

In the present study the feasibility of the strategy to target drugs to the macrophages via the oral route was tested in rats. AZT was chosen as a model drug, because at the beginning of the study it was the only approved anti-AIDS drug and because it was commercially available in radioactive form. AZT-nanoparticles and an AZT control solution prepared identically to the nanoparticles but without monomer were administered by gavage to rats. The radioactivity was measured in reticuloendothelial cell containing organs as well as in organs containing no reticuloendothelial cells. The body distribution of free and nanoparticle-bound AZT was compared.

2. Materials and methods

2.1. Synthesis and preparation of nanoparticles and control solutions

Nanoparticles were produced by emulsion polymerization after addition of 100 μl *n*-hexylcyanoacrylate monomer (Sichel Werk, Hannover, Germany), as well as 20 mg bis(2-ethylhexyl) sulfosuccinate sodium as an emulsifier (DOSS) (FLUKA, Neu-Ulm, Germany) to 10 ml of an aqueous solution of 0.01 N HCl containing 10 mg [^{14}C]AZT (SIGMA, Deisenhofen, Germany) with an activity of 274 μCi. The monomer was added dropwise to the solution under magnetic stirring (600 rpm). After 96 h polymerization was completed by neutralisation with 100 μl 1 N sodium hydroxide. After another 60 min 5% (w/v) of mannitol (Phoenix, Hanau, Germany) was added to the suspension. This suspension was then administered.

Control solutions with the same [^{14}C]AZT and DOSS content were prepared identically, but without monomer.

2.2. Determination of drug loading

An aliquot of 185 μl of the suspension was centrifuged for 30 min at 100 000 g with an Air Fuge (Beckman Instruments, München, Germany). Unbound AZT was assayed by HPLC in the supernatant. HPLC analysis was performed using a Perkin Elmer Series II pump with autosampling system ISS 100 and integrator LCI 100, (Perkin Elmer, Langen, Germany). The analytical column (LiChroCART 125-4) was filled with LiChrospher 100 RP-18 (5 μm), the guard column was a LiChroCART 4-4 filled with the same material (Merck, Darmstadt, Germany). The mobile phase consisted of 15% (v/v) acetonitrile, 0.12 mol phosphate buffer, pH 6.2 and 14.4 mmol triethylamine with a flow rate of 1.0 ml/min. These chemicals were analytical grade. UV detection was carried out at a wavelength of maximum absorbance, 265 nm, with a Lambda-Max 480 UV detector (Waters, Eschborn, Germany). The analysis showed that 51.5 ± 4.9% of the AZT was bound to the particles.

2.3. Particle size and zeta potential

The particle size was measured by laser light scattering using a BI 200 SM Goniometer with digital correlator and PC (Brookhaven, Holtsville, NY). The average diameter of the particles was 230 ± 20 nm.

The zeta potential was determined with a Lazer Zee Meter Mod. 501 (Penkem, Bedford Hills, NY). The nanoparticle preparation showed a zeta potential of − 51.6 ± 3 mV.

2.4. Drug liberation

Drug release from the nanoparticles was examined using Higuchi side-by-side diffusion cells (Fischer OHG, Frankfurt, Germany). A dialysis membrane consisting of cellulose acetate (Medicell, London, UK) with a molecular weight cut-off of 12 000–14 000 was used to separate the chambers. Prior to the experiment the membrane was washed with distilled water for 1 h. As release medium isotonic saline solution was chosen. 100 μl of the nanoparticle suspension/drug solution was added to 1900 μl of saline solution in the donor side of the diffusion cells. The receiver chambers were filled with 2 ml saline solution. After regular periods of time samples of 100 μl were taken out of the receiver side and replaced by fresh saline solution.

2.5. Animal experiments

Authorization for the animal experiments was given by the Regierungspräsidium Darmstadt, Germany under the registration number II 17a–19c 20/15-F 116/05.

The animals were kept under standard conditions, with free access to water and food. Nanoparticle preparations and AZT solutions were administered by gavage. For each preparation and each sampling time point, four rats, two male and two female (Wistar Unilever rats, Harlan Winkelmann, Borchen, Germany), of a body weight between 180 and 220 g were treated with a single dose of 3.3 mg AZT (90.1 μCi)/kg body weight. After dosing the animals were kept in individual cages until they were sacrificed using CO_2, i.e. after 30 min, 1, 2, 4, 6 and 8 h, respectively.

The elimination pathways of the AZT-nanoparticles and of the control solution were studied by collecting faeces and urine over 24 h from rats (two male and two female) using metabolic cages (Hoechst, Frankfurt, Germany).

2.6. Determination of ^{14}C-radioactivity

The animals were dissected and a portion of each organ was removed. Every sample was accurately weighed into a glass vial. The weight of the organs was estimated from the total body weight employing the following percentages: GI-tract 6.19, bone marrow 1.3, muscles 45 and blood 5 (Dr. Maas, Hoechst, Frankfurt, Germany). After dissolving the tissue in 1 ml BTS 450 tissue solubilizer at 50°C (Beckman, München, Germany), 500 μl of hydrogen peroxide solution (30% v/v) was added until the colour disappeared. After addition of 100 μl glacial acetic acid and 10 ml liquid scintillation cocktail (Ready Organic, Beckman, München, Germany) the samples were stored for 1 week in darkness before radioactivity was determined in a scintillation counter (model LS 1801, Beckman, München, Germany).

3. Results and discussion

The choice of a suitable model for the estimation of the drug release from nanoparticles is always problematic. A major problem with conventional separation procedures such as ultrafiltration or ultracentrifugation represents the time period between sampling and content determination required for the separation of free drug from nanoparticle-bound drug during which drug release continues. In the present experiments dynamic dialysis was chosen for the separation of free drug from nanoparticle-bound drug. This separation was achieved by a molecular weight cut off of the dialysis membrane of 12 000–14 000. In Fig. 1, the AZT transport is shown for AZT bound to nanoparticles and for free AZT. For drug bound to nanoparticles the release into

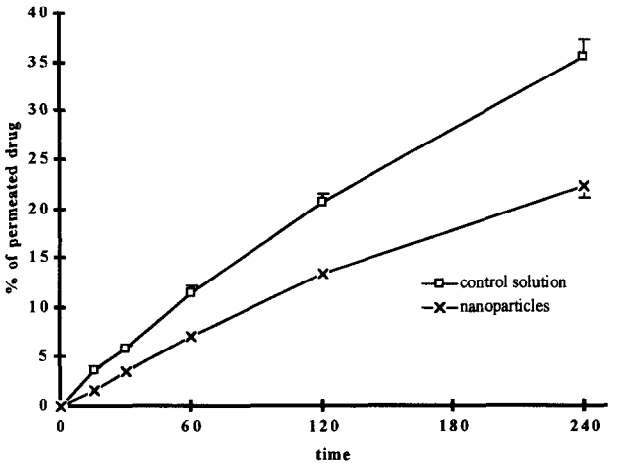

Fig. 1. Drug liberation of [^{14}C]AZT from nanoparticles compared with free [^{14}C]AZT in a Higuchi side-by-side diffusion cell. The drug liberation from the nanoparticle preparation was delayed compared to the free [^{14}C]AZT.

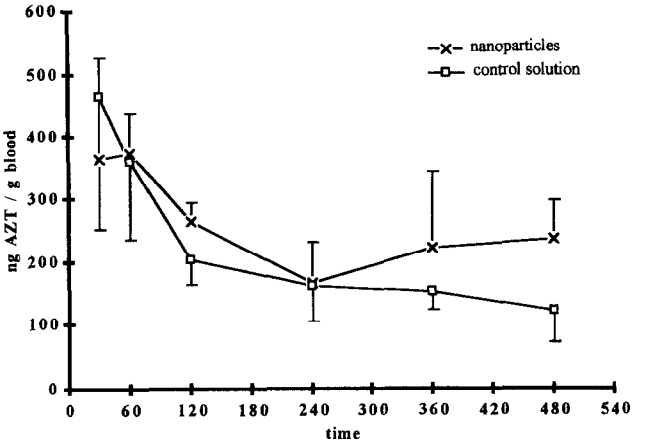

Fig. 2. Blood concentration of [^{14}C]AZT following oral dosing of [^{14}C]AZT bound to nanoparticles and a [^{14}C]AZT control solution.

the receiver chamber was slower compared to the free AZT, indicating a delayed drug liberation by the nanoparticles.

The blood level versus time curves of the orally administered [^{14}C]AZT nanoparticle preparation and the [^{14}C]AZT control solution are shown in Fig. 2. The highest blood level was observed after the first experimental point, 30 min with the solution and after 60 min with the nanoparticles. Similar peak ^{14}C-label concentrations were found in all organs except in the bone marrow and the lymph nodes (Table 1). In these organs the C_{max} of the nanoparticle preparation appeared at 2 or 4 h respectively. The delayed absorption of the [^{14}C]AZT bound to nanoparticles in comparison to the solution was coupled with an increase of the AUC of the ^{14}C-label by 20% in the blood (Table 2). Similar increases in bioavailability due to binding of the drug to nanoparticles were previously reported by Maincent et al. for vincamine [19], Beck et al. for avarol [20] and Damgé et al. for lipiodol [12]. The bioavailability was 1.75-fold higher with vincamine, 8–9-fold higher with avarol and 2.5 higher with lipiodol compared to the respective control solutions. In the present study a second increase in the ^{14}C-label blood levels between 4–8 h was observed after the first peak (Fig. 2). This effect might be caused by a prolonged absorption of the drug due to bioadhesion of the nanoparticles to the gut. This assumption would be in agreement with earlier findings of Pimienta et al. who showed an adhesion of poly (hydroxypropyl-methacrylate) nanoparticles to the intestinal mucosa [21]. Slow degradation of these particles in the gut by esterases [22] could be responsible for the delayed release and the observed increase in the [^{14}C]AZT-label concentration after 4 h.

The most pronounced difference between [^{14}C]AZT label bound to nanoparticles and the free [^{14}C]AZT label was found in the liver (Fig. 3). The AUC was increased by the nanoparticles by 30% (Table 2) com-

Table 1
Concentration of [^{14}C]AZT (ng ^{14}C-AZT/g organ) after oral administration of AZT bound to nanoparticles and an AZT control solution

Organ	Time (min)											
	30		60		120		240		360		480	
	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.	Mean	±S.D.
[^{14}C]AZT bound to nanoparticles (ng/g organ)												
Blood	366	161	375	65	265*	30	166	66	224	122	236*	62
Bone marrow	410	234	522	268	576*	30	293	120	221	57	145	79
Brain	118	75	133	35	77*	39	80*	13	72*	12	59*	4
Heart	394	179	434	100	244*	29	136	65	98	18	72	13
Kidneys	1892	2034	2315	2042	1073*	249	400	64	274*	48	173	42
Liver	872	449	921	250	784*	135	573	88	448*	72	320*	68
Lungs	461	187	526	89	312*	41	170	31	136*	14	103*	13
Lymphnodes	1315	92	1643	574	657	346	2547*	2042	1509	1617	744*	270
Muscles	341	271	401	108	224	27	111	12	83	14	77	19
Ovaries	551	405	898	718	219	44	142	18	147	28	138	43
Spleen	413*	158	873	563	397	77	267	85	184*	43	152*	27
Testicles	92	8	255	38	468	283	118	25	87	13	64	8
[^{14}C]AZT control solution (ng/g organ)												
Blood	467	215	361	123	204*	39	161	55	152	28	122*	49
Bone marrow	722	414	464	189	368*	30	239	93	194	74	164	64
Brain	116	61	100	28	57*	25	62*	10	44*	5	44*	6
Heart	365	322	437	159	187*	38	133	37	80	28	63	11
Kidneys	3694	1829	2274	1047	779*	197	518	240	175*	48	152	45
Liver	1418	614	1113	556	437*	92	375	108	234*	52	214*	65
Lungs	738	424	552	196	218*	50	156	61	90*	16	80*	18
Lymphnodes	2415	1786	1516	927	556	111	616*	173	759	367	438*	107
Muscles	564	320	396	150	179	32	122	33	69	13	59	19
Ovaries	645	184	434	62	165	32	144	50	113	48	85	21
Spleen	982*	373	771	517	365	177	212	73	130*	17	100*	27
Testicles	293	267	213	85	210	102	130	16	65	10	61	24

* Statistically significant ($P<0.05$).

pared to the control solution. The differences between the two preparations amounted to a factor of 1.8 after 2 h and to 1.5 after 8 h. In the other reticuloendothelial cell containing organs such as the lungs, spleen or bone marrow the nanoparticles also caused higher [^{14}C]AZT label concentrations after 1 hour compared to the control solution (Table 1). These differences, however, were in general not statistically significant. Nevertheless, the question arises, what is the mechanism for the enhanced transport to these organs? Le Ray et al. [23] reported a transport of nanoparticles to the liver after single oral dosing. In other studies [24] particles also were found in spleen and lymph. The quantity of gut uptake of nanoparticles and their translocation to organs seems to depend strongly on their size [25] and on their apparent hydrophobicity [26]. Smaller particle sizes and more hydrophobic particles increase uptake. In general, gut uptake of intact particles is rather low [25]. The improvements in bioavailability and in transport to the organs, therefore, have to be explained by different mechanisms. Leroux et al. [27] who observed a similar favourable peroral bioavailability with a novel HIV-1 protease inhibitor bound to Eudragit L 100-55 nanoparticles, suggested that an increased surface area, combined with a rapid dissolution of the drug and an optimal dispersion due to the nanoparticulate character may be responsible for the excellent performance of the nanoparticles. However, it must be kept in mind that these factors do not provide a satisfactory explanation for the superior bioavailability, nor for the greater transport to the reticuloendothelial cell containing organs of the nanoparticles in comparison with the solution formulation. In fact, the solution formulation should meet the parameters brought into discussion by Leroux et al. better than the nanoparticles. An alternative explanation is that nanoparticles would allow a more intimate and prolonged contact of the bound drug with the absorptive cells in the gut due to their bioadhesive properties. It has to be mentioned in this context, that considerably increased transport through an artificial dialysis membrane was already seen in vitro with [^{75}Se]norcholesterol bound to poly(cyanoacrylate) nanoparticles comparable to those used in the present study [28]. Although the mechanism of this type of transport and the contribution of bioadhesion still is not clear, we assume that similar mechanisms may

Table 2
Pharmacokinetic parameters of peroral [^{14}C]AZT nanoparticles and a [^{14}C]AZT control solution

Organ	Preparation	AUC_{0-480} ng min ml^{-1}	$AUC_{0-\infty}$ ng min ml^{-1}	AUC_{rem} ng min ml^{-1}	C_{max}	t_{max}	$t_{1/2}$
Blood	Nanoparticles	111 980	—	—	375	60	—
Blood	Control solution	92 798	186 594	50	467	30	525
Liver	Nanoparticles	278 352	410 600	32	921	60	283
Liver	Control solution	213 953	307 442	30	1418	30	318
Kidney	Nanoparticles	336 549	386 527	13	2315	60	198
Kidney	Control solution	361 230	387 409	7	3694	30	139

AUC_{0-480}, area under the curve between 0–480.
$AUC_{0-\infty}$, area under the curve between 0–∞.
AUC_{rem}, % of calculated AUC after t_{480}.
$t_{1/2}$, elimination half time.

contribute to the improved bioavailability observed with [^{14}C]AZT. The higher uptake by liver, spleen and lymph nodes on the other hand may be due to an enhanced lymphatic uptake as reported by Maincent et al. and Jani et al. [24,25,29].

Another organ that is relevant for AIDS therapy is the brain since the HI-virus is able to infect this organ [30]. In addition, in the brain cells specific transport mechanisms are located [31], that actively eliminate AZT from these cells. Consequently, an increase of the AZT concentration in the brain would be an important advantage for AIDS therapy. The [^{14}C]AZT label concentration in this organ (Fig. 4) was about 33% higher after 1 hour with nanoparticles as compared to the solution. This trend persisted for the whole observation period. Therefore, peroral nanoparticles may be considered to improve delivery of AZT to the brain.

The elimination pathway of [^{14}C]AZT was examined using metabolic cages. The control solution was eliminated via the kidneys by 96 ± 2.8% which corresponds to literature data [32]. In contrast, [^{14}C]AZT label bound to nanoparticles was eliminated via the faeces by 62 ± 3.7%. This high amount of drug in the faeces may be explained in two ways. First, the absorption of [^{14}C]AZT label bound to nanoparticles might not be complete. Second, the elimination pathway of [^{14}C]AZT label was changed by the particles as it was already reported for intravenously injected [^{14}C]AZT preparations [3]. However, since the concentrations of the ^{14}C-label in the blood are similar for both preparations, nanoparticles and free drug, and since the concentrations in most organs are slightly higher in most cases with the nanoparticles between 60–480 min (end of experimental frame), an incomplete gut uptake cannot be postulated and cannot be the reason for the high faecal elimination. In addition, the kidneys AUC's for [^{14}C]AZT following administration of nanoparticles and the control solution were nearly the same (Table 2), indicating that the same drug amount passed through this organ over the 8 hours of the study. Nanoparticle-bound radioactivity, however, obviously was not eliminated to the same extent by the kidneys as the free drug. The nanoparticles, in contrast, seem to enhance biliary excretion as already observed by Nefzger et al. [6].

In the present study the beneficial properties of nanoparticles as drug carrier for an antiviral therapy are apparent: The AUC in the organs that are mainly infected by the HI-virus, namely the reticuloendothelial cell containing organs, the blood, and the brain was

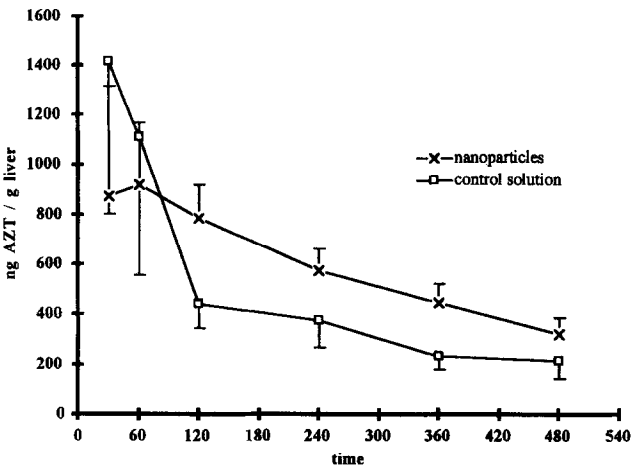

Fig. 3. Liver concentration of [^{14}C]AZT following oral dosing of [^{14}C]AZT bound to nanoparticles and a [^{14}C]AZT control solution.

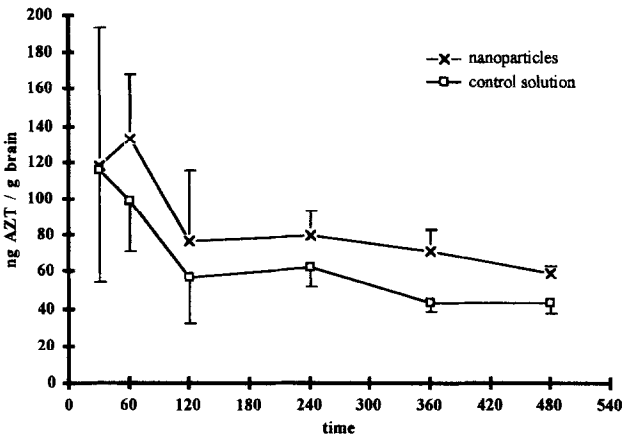

Fig. 4. Brain concentration of [^{14}C]AZT following oral dosing of [^{14}C]AZT bound to nanoparticles and a [^{14}C]AZT control solution.

increased when nanoparticles were used perorally. Consequently, nanoparticles represent a very promising drug delivery system for AZT and for AIDS therapy in general. These properties also might be advantageous for other poorly soluble drugs or for drugs that cannot cross the blood-brain-barrier.

Acknowledgements

The authors gratefully acknowledge the BMFT/BGA for sponsoring this work through the 'Frankfurter AIDS Schwerpunkt' (Grant FKZ III 004-89).

References

[1] S. Roy, M.A. Wainberg, Role of the mononuclear phagocyte system in the development of acquired immunodeficiency syndrome (AIDS), J. Leukocyte Biol. 43 (1989) 91–97.
[2] M.S. Meltzer, D.R. Skillman, D.L. Hoover, B.D. Hanson, J.A. Turpin, D.C. Kalter, H.E. Gendelman, Macrophages and the human immunodeficiency virus, Immunol. Today 11 (1990) 217–223.
[3] R. Löbenberg, J. Kreuter, Macrophage-targeting of AZT: a promising strategy for AIDS therapy, AIDS Res. Hum. Retroviruses 12 (1996) 1709–1715.
[4] J. Kreuter, U. Täuber, V. Illi, Distribution and elimination of poly(methyl-2-[^{14}C]methacrylate) nanoparticle radioactivity after injection in rats and mice, J. Pharm. Sci. 68 (1979) 1443–1447.
[5] L.D. S.S. Illum, C.G. Wilson, N.W. Thomas, M. Frier, J.G. Hardy, Blood clearance and organ deposition of intravenously administered colloidal particles. The effects of particle size, nature and shape, Int. J. Pharm. 12 (1982) 135–146.
[6] M. Nefzger, J. Kreuter, R. Voges, E. Liehl, R. Czok, Distribution and elimination of polymethyl-methacrylate nanoparticles after peroral administration to rats, J. Pharm. Sci. 73 (1984) 1309–1311.
[7] A.T. Florence, A.M. Hillery, N. Hussain, P.U. Jani, Factors affecting the oral uptake and translocation of polystyrene nanoparticles: histological and analytical evidence, J. Drug Targeting 3 (1995) 65–70.
[8] G.M. Hodges, E.A. Carr, R.A. Hazzard, C. O'Reilly, K.E. Carr, A commentary on morphological and quantitative aspects of microparticle translocation across the gastrointestinal mucosa, J. Drug Targeting 3 (1995) 57–60.
[9] P.U. Jani, D.E. McCarthy, A.T. Florence, Nanosphere and microsphere uptake via Peyer's patches: observation of the rate of uptake in the rat after single oral dose, Int. J. Pharm. 86 (1992) 239–246.
[10] A.M. Hillery, P.U. Jani, A.T. Florence, Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles, J. Drug Targeting 2 (1994) 151–156.
[11] J. Kreuter, V. Müller, K. Munz, Quantitative and microautoradiographic study on mouse intestinal distribution of polycyanoacrylate nanoparticles, Int. J. Pharm. 55 (1989) 39–45.
[12] C. Damgé, M. Aprahamian, G. Balboni, A. Hoeltzel, V. Andrieu, J.P. Devissaguet, Polyalkylcyanoacrylate nanocapsules increase the intestinal absorption of a lipophilic drug, Int. J. Pharm. 36 (1987) 121–125.
[13] J. McCullough, G. Hodges, G. Dickson, A. Yarwood, K. Carr, A morphological and microanalytical investigation onto the uptake of particulate iron across the gastrointestinal tract of rats, J. Submicrosc. Cytol. Pathol. 27 (1995) 119–124.
[14] D. O'Hagan, Intestinal translocation of particulates: implication for drug and antigen delivery, Adv. Drug Deliv. Rev. 5 (1990) 265–285.
[15] G. McLeod, S. Hammer, Zidovudine: five years later, Ann. Intern. Med. 117 (1992) 487–501.
[16] A. Bender, V. Schäfer, A. Steffan, C. Royer, J. Kreuter, H. Rübsamen-Waigmann, H.V. von Briesen, Inhibition of HIV in vitro by antiviral drug-targeting using nanoparticles, Res. Virol. 145 (1994) 215–220.
[17] P. Schölmerich, E. Mutschler, G. Thews, Saquinavir, Proteasehemmer als neue Kombinationspartner, Arzneimitteltherapie 14 (1996) 58–60.
[18] A. Bender, H. von Briesen, J. Kreuter, I.B. Ducan, H. Rübsamen-Waigmann, Efficiency of nanoparticles as carrier system for antiviral agents in human immunodeficiency virus-infected human monocytes/macrophages in vitro, Antimicrob. Agents Chemother. 40 (1996) 1467–1471.
[19] P. Maincent, R.L. Verge, P. Sado, P. Couvreur, J.P. Devissaguet, Disposition kinetics and oral bioavailability of Vincamine loaded polyalkylcyanoacrylate nanoparticles, J. Pharm. Sci. 75 (1986) 955–958.
[20] P.H. Beck, J. Kreuter, W.E.G. Müller, W. Schatton, Improved peroral delivery of avarol with polybutylcyanoacrylate nanoparticles, Eur. J. Biopharm. 40 (1994) 134–137.
[21] C. Pimienta, V. Lenaerts, C. Cadieux, P. Raymond, J. Jushasz, M.-A. Simard, C. Jolicoeur, Mucoadhesion of hydroxypropylmethacrylate nanoparticles to rat intestinal ileal segments in vitro, Pharm. Res. 7 (1990) 49–53.
[22] D. Scherer, J.R. Robison, J. Kreuter, Influence of enzymes on the stability of polybutylcyanoacrylate nanoparticles, Int. J. Pharm. 101 (1994) 165–168.
[23] A.M. Le Ray, M. Vert, J.C. Gautier, J.P. Benoit, Fate of [^{14}C]poly(DL-lactide-co-glycolide) nanoparticles after intravenous and oral administration to mice, Int. J. Pharm. 106 (1994) 201–211.
[24] P. Jani, W. Halbert, J. Langride, A.T. Florence, The uptake and translocation of latex nanospheres and microspheres after oral administration to rats, J. Pharm. Pharmacol. 41 (1989) 809–812.
[25] P. Jani, G.W. Halbert, J. Langridge, A.T. Florence, Nanoparticle uptake by rat gastrointestinal mucosa: quantification and particle size dependency, J. Pharm. Pharmacol. 42 (1990) 821–826.
[26] J.H. Eldridge, C.J. Hammond, J.A. Meulbroek, J.K. Staas, R.M. Gilley, T.R. Tice, Controlled vaccine release in the gut-associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches, J. Control. Release 11 (1990) 205–214.
[27] J.-C. Leroux, R.M. Cozens, J.L. Roesel, B. Galli, F. Kubel, E. Doelker, R. Gurny, Pharmacokinetics of a novel HIV-1 protease inhibitor incorporated into biodegradable or enteric nanoparticles following intravenous and oral administration to mice, J. Pharm. Sci. 84 (1995) 1387–1391.
[28] J. Kreuter, S.N. Mills, S.S. Davies, C.G. Wilson, Polybutylcyanoacrylate nanoparticles for the delivery of [^{75}Se]norcholesterol, Int. J. Pharm. 16 (1983) 105–113.
[29] P. Maincent, P. Thouvenot, C. Amicabile, M. Hoffman, J. Kreuter, P. Couvreur, J.P. Devissaguet, Lymphatic targeting of polymeric nanoparticles after intraperitoneal administration in rats, Pharm. Res. 9 (1992) 1534–1539.
[30] G. Christofinis, L. Papadaki, Q. Sattentau, R.B. Ferns, R. Tedder, HIV replicates in cultured human brain cells, AIDS 1 (1987) 229–234.
[31] K. Dykstra, A. Arya, D. Arriola, P. Bungay, P. Morrion, R.L. Dedrick, Microdialysis study of Zidovudine (AZT) transport on rat brain, J. Pharmacol. Exp. Ther. 27 (1993) 1227–1236.
[32] J.E.F. Reynolds (Ed.), Antiviral agents, Martindale, vol. 30, Pharmaceutical Press, London, 1993, pp. 560–563.