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Enhanced transport of nanoparticle associated drugs through natural and artificial membranes—a general phenomenon?

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Abstract

The transport of nanoparticle associated drugs, [^{75}Se]norcholestenol, captopril, methylene blue, hydrocortisone, doxorubicin, and dalargin was determined by permeability measurements in two chamber side by side diffusion cells using cellulose acetate, silicone rubber, pig small intestine, or hairless mice skin as membranes. Solutions of free drugs served as controls. The permeabilities depended on the physico chemical properties of the drugs which governed both, drug interaction with the nanoparticles as well as with the membranes. Consequently, the influence of dilution of the nanoparticle or free drug preparations on permeabilities was complex. With the exception of [^{75}Se]norcholestenol the permeabilities were higher with free drugs than after binding to nanoparticles. The permeabilities of the membranes decreased in the order cellulose acetate, pig small intestine, silicone rubber, and hairless mouse skin. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Nanoparticles; [75Se]norcholestenol; Captopril; Methylene blue; Hydrocortisone; Doxorubicin; Dalargin

1. Introduction

A couple of years ago Kreuter et al. (1983) observed that the [^{75}Se]norcholestenol delivery across a cellulose acetate membrane into the receiver chamber of a two-chamber dialysis cell was much more efficient with nanoparticles than with a solution. In these experiments the steroidal drug [^{75}Se]norcholestenol was incorporated into polybutylcyanoacrylate nanoparticles, the control was a micellar solution of this drug. These authors then followed the body distribution kinetics of these two dose forms after intravenous and intramuscular administration over 30 days with a γ-camera in rabbits. They observed that the

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[^{75}Se]norcholestenol radioactivity remained much longer in the body with the nanoparticles, although body distribution experiments in rats by Grislain et al. (1983) and Couvreur et al. (1986) had clearly shown that the ^{14}C-labelled polycyanoacrylate nanoparticle polymer matrix was degraded and excreted rapidly, i.e. in a few days. These findings indicated that the nanoparticles delivered the [^{75}Se]norcholestenol to tissues in the body much more efficiently than the micellar solution similarly to the above described in vitro events. The tissues in the bodies of the rabbits then acted as a reservoir for the drug and led to prolonged residence times of the drug.

Some years later, Scherer (1992) made similar observations: a more efficient delivery of captopril with nanoparticles compared to a solution across a number of membranes.

In this context membrane transport experiments by Kreuter et al. (1981) with liposomes also are of interest. These authors found that aqueous boundary layers on the surface of the membranes but also on the surface of colloidal carriers strongly influence the interaction and the transport of drugs incorporated into the carriers across these membranes. Accordingly, by a possible reduction of the retarding influence of these layers, colloidal carriers such as liposomes and nanoparticles have a potential to enhance drug delivery by mediating a more direct or prolonged contact of the carrier and bound drug than would be possible with single disperse molecules in a solution. Especially hydrophobic drugs could benefit from the avoidance of an aqueous boundary layer by transport from a hydrophobic environment directly into another hydrophobic environment.

These observations taken together led to the questions: is this phenomenon of enhanced drug transport across membranes a general or a unique event, and to what sorts of drugs does it apply? In addition, do boundary layers as observed with liposomes also play a role in this phenomenon? To answer these questions a wide range of nanoparticle bound drugs was employed in our present study and the transport across a variety of membranes was investigated and compared with the respective drug solutions. Side by side diffusion chambers were used, and three different drug as well as three different carrier concentrations were employed. The drug preparations were optimized for drug binding and, therefore, different surfactant combinations had to be used.

2. Materials and methods

2.1. Chemicals

Butyl-2-cyanoacrylate was obtained from Sichel-Werke, Hannover, Germany; polysorbate 80 from ICI, Middlesbrough, UK; poloxamer 188 from Erbslöh, Düsseldorf, Germany; dextran 70 000, captopril, methylene blue, and 6,6′-dinitro-3,3′-dithio-benzioc acid from Sigma, Deisenhofen, Germany; 1 N hydrochloric acid and 1 N sodium hydroxide from Merck, Darmstadt, Germany; [^{75}Se]norcholestenol (Scintadren®) from Amersham Buchler, Braunschweig, Germany; hydrocortisone from Synopharm, Barsbüttel, Germany; doxorubicin from Pharmacia, Erlangen, Germany; and dalargin from Bachem Biochemica, Heidelberg, Germany.

2.2. Preparation and characterisation of nanoparticles

2.2.1. [^{75}Se]norcholestenol nanoparticles

Polysorbate 80 (1.6%, 1 ml) containing a commercial micellar solution of [^{75}Se]norcholestenol (Scintadren®, 200 μCi (7.4 MBq)) was added to 10 ml 0.1 N HCl containing 50 μl polysorbate 80. Butylcyanoacrylate (bca) (100 μl) was added and the mixture stirred for 4 h, resulting in the formation of polybutylcyanoacrylate nanoparticles. This mixture was neutralized with 1 ml 1 N NaOH and stirred for 24 h (Kreuter et al., 1983).

Aliquots of the stock nanoparticle preparation 1AZ/1NP were diluted 1:2 and 1:10 in order to obtain the preparations 0.5AZ/0.5NP and 0.1AZ/0.1NP, keeping the ratio between drug and nanoparticles constant.

Additionally, the drug-nanoparticle ratio was varied by producing two more nanoparticle batches with drug to nanoparticle ratios of 1:2 and 1:10 (0.5AZ/1NP and 0.1AZ/1NP).

2.2.2. Captopril nanoparticles

Captopril polybutylcyanoacrylate nanoparticles were produced by incorporative as well as by adsorptive loading. Incorporative loading was performed as described above for [^{75}Se]norcholestenol nanoparticles using a solution of 0.5 g poloxamer 188 and 0.250 g captopril in 49 ml 0.01 N HCl and adding 0.5 ml butylcyanoacrylate. Neutralization was performed with 4.9 ml 0.1 N NaOH.

Adsorptive loading was performed by dissolution of 0.5 g poloxamer 188 in 49 ml 0.01 N HCl, addition of 0.5 ml butylcyanoacrylate, and 4 h stirring. The pH value was then increased to 3.5 with 0.1 N NaOH and 0.250 g captopril was added and adsorbed on the nanoparticles by further stirring for 24 h. After this pH neutralisation was performed with 0.1 N NaOH to yield the 1AZ/1NP preparation.

Aliquots of this preparation were diluted 1:2 and 1:10 to obtain the preparations 0.5AZ/0.5NP and 0.1AZ/0.1NP, respectively. The preparations 0.5AZ/1NP and 0.1AZ/1NP were produced by addition of 0.125 g and 0.025 g instead of 0.250 g captopril (see above) to the nanoparticles.

2.2.3. Methylene blue nanoparticles

To a solution of 0.5 g poloxamer 188 and 0.200 g methylene blue in 49 ml 0.01 N HCl 0.5 ml butylcyanoacrylate were added as described above. The mixture was stirred for 4 h, then neutralized with 4.9 ml 0.1 N-NaOH and further stirred for 24 hours.

The preparations of 0.5AZ/0.5NP and 0.1AZ/0.1NP again were produced by dilution of the 1AZ/1NP stock preparation; the preparations 0.5AZ/1NP and 0.1AZ/1NP were produced employing 0.100 or 0.020 g methylene blue.

2.2.4. Hydrocortisone nanoparticles

Empty polybutylcyanoacrylate nanoparticles were produced in the same way as described for captopril nanoparticles. After neutralisation 0.150 g hydrocortisone under gentle heating dissolved in 2.5 g polysorbate 80 was added and the mixture was stirred for 24 h.

The preparations 0.5AZ/0.5NP, 0.1AZ/0.1NP, 0.5AZ/1NP and 0.1AZ/1NP were produced as described above.

2.2.5. Doxorubicin nanoparticles

Lyophilized drug preparation (240 mg) (Adriblastin®: 40 mg doxorubicin hydrochloride and 200 mg lactose·1 H_2O) provided by Pharmacia, Erlangen, Germany, were dissolved in the 30 ml original aqueous solution which contained 5% glucose, 1% dextran 70 000 and 0.5% citric acid. Under stirring 614 mg butylcyanoacrylate monomer was slowly added and polymerization was performed by stirring this mixture for 6 h.

The preparations 0.5AZ/0.5NP and 0.1AZ/0.1NP were produced by dilution of the original 1AZ/1NP stock suspension, 0.5AZ/1NP and 0.1AZ/1NP were produced using the appropriate lower amounts of doxorubicin.

2.2.6. Dalargin nanoparticles

Empty polybutylcyanoacrylate nanoparticles were produced as described for captopril. After neutralization of the reaction mixture with 0.1 N NaOH, 0.15 g dalargin was added to a 50 ml empty nanoparticle suspension and stirring was continued for a further 24 h.

The preparations 0.5AZ/0.5NP and 0.1AZ/0.1NP were produced by dilution of the 1AZ/1NP stock suspension and 0.5AZ/1NP and 0.1AZ/1NP were produced by addition of the appropriate lower amounts of dalargin to the empty nanoparticles.

2.3. Particle size determination

The particle size of the nanoparticles was determined by photon correlation spectroscopy after dilution of 25 μl of the nanoparticle suspension with 10 ml filtered (cellulose membrane filter, 0.22 μm, Satorius, Göttingen, Germany) distilled water using a Goniometer BI-200 SM, Version 2.0 (Brookhaven Instruments, Holtsvill, NY, USA) equipped with a helium–neon laser, 30 mW (Melles Griot, Cincinatti, USA).

2.4. Drug loading

The nanoparticle suspensions were centrifuged at $100\,000 \times g$ for 30 min (Airfuge, Beckman, München, Germany). The supernatants or dialysis experiment samples were assayed in the case of

[^{75}Se]norcholestenol in a γ counter (Clini-Gamma, Type 1272, LKB-Wallac, Turku, Finland) or in a spectrophotometer (Hitachi, Tokyo, Japan) at the following wavelenghts: captopril, 412 nm; methylene blue, 668 nm; hydrocortisone, 299 nm; doxorubicin, 495 nm; and dalargin, 220 nm. With captopril Ellmann's reagent (6,6′-dinitro-3,3′-dithiobenzioc acid, 39.5 mg dissolved in 100.0 ml Ringer-bicarbonate-buffer, pH 7) was added to the captopril solutions in the supernatant or to the dialysis samples and left for at least 5 min before spectrometric determination.

2.5. Thin layer chromatography of captopril nanoparticles

Captopril nanoparticles were produced by two polymerization methods, firstly in the presence (incorporation) of captopril and secondly by adsorption of captopril onto previously polymerized empty nanoparticles. In order to obtain rapid information about the difference between the two mixtures, the nanoparticle preparations were subjected to thin layer chromatography. For this purpose the nanoparticle suspensions were mixed 1:1 with acetone, and about 50 μl of this mixture or solution of free drug in acetone was applied to thin layer chromatography plates (Analtech Silica Gel G Platte, Merck, Darmstadt, Germany). Toluene–acetic acid 3:1 was used as an eluent. After 80 min the plates were dried and sprayed with 39.5 mg 6,6′-dinitro-3,3′-dithiobenzioc acid in 100 ml methanol.

2.6. Dialysis experiments

Certain amounts, 60 μl for [^{75}Se]norcholestenol, 3.5 ml for captopril, 1.5 ml for methylene blue, and 7.0 ml for hydrocortisone, doxorubicin, and dalargin, of the drug nanoparticle suspensions (AZ/NP) or solutions of the same drugs in the same concentrations (AZ) were added to the donor compartment of two-compartment diffusion cells (half-cell volume 7.5 ml). The cells contained phosphate-buffered saline (pH 7.4) in both compartments, separated by a natural or artificial dialysis membrane and were maintained at 37.0. $\pm$ 0.1°C. Samples of 100 μl were drawn after an initial equilibration time of 5 min from the donor as well as from the receiver compartments. Every 30 min over a period of 4 h 100 μl samples were drawn from the receiver compartment and assayed as described for drug loading without centrifugation. After sampling the sample volume extracted was replaced by the same volume of phosphate buffer. All dialysis experiments were repeated six times.

The following membranes were used: cellulose acetate (Dialysis Tubing-Visking, Size 9-36/32'', cut off 12 000–14 000, Medicell International, London, UK); silicone membrane, 175 μm thickness (Freudenberg, Weinheim, Germany); small intestine from pigs obtained from the slaughter house, transported immersed in Ringer solution on ice and used within 45 min after sacrifice, rinsed about several times with a total of approximately 500 ml sodium chloride isotonic solution; and full thickness dorsal and abdominal hairless mice skin from over 120 days old hairless mice (breed HsdOla: MF1-hr/hr, Harlan Winkelmann, Borchen, Germany), subcutaneous fat was scratched off with a scalpel.

2.7. Permeability coefficients

The permeability coefficients P were calculated according to Eq. (1),

$$P\ (\mathrm{cm/s})\ 1 \times 10^{-6} = \frac{V(\mathrm{d}C/\mathrm{d}t)}{A \times \Delta C} \quad (1)$$

where V is the half-cell volume, $\mathrm{d}C/\mathrm{d}t$ is the concentration differential of drug in the receiver chamber after 70 min, A the diffusional area of the membrane, and ΔC the concentration difference between donor and receiver chamber after 5 min.

3. Results and discussion

3.1. Particle size

The diameters of unloaded nanoparticles and those of the drug associated nanoparticles were measured directly after their production. The average hydrodynamic diameter of unloaded poly-

Table 1
Average particle size and drug loading of polybutylcyanoacrylate nanoparticles ($n = 4$)[a]

	Average particle size (nm)	Average drug loading [% (G/G)]
[^{75}Se]norcholestenol polybutylcyanoacrylate nanoparticles		
Incorporative drug loading		
1AZ/1NP	257 ± 17.68	43.98 ± 5.1
0.5AZ/1NP	225 ± 35.36	63.35 ± 3.4
0.1AZ/1NP	200 ± 28.2	72.28 ± 5.5
Unloaded NP	115 ± 21.2	0
Captopril polybutylcyanoacrylate nanoparticles		
Incorporative drug loading		
1AZ/1NP	260 ± 14.14	95.10 ± 1.96
0.5AZ/1NP	220 ± 15.26	96.46 ± 1.74
0.1AZ/1NP	190 ± 14.3	97.78 ± 1.35
Unloaded NP	115 ± 21.21	0
Adsorptive drug loading		
1AZ/1NP	170 ± 14.14	81.9 ± 1.27
0.5AZ/1NP	153 ± 10.6	85.2 ± 1.04
0.1AZ/1NP	145 ± 7.07	90.9 ± 2.3
Methylene blue polybutylcyanoacrylate nanoparticles		
Incorporative drug loading		
1AZ/1NP		88.6 ± 4.4
0.5AZ/1NP		64.1 ± 4.3
0.1AZ/1NP		48.1 ± 2.6
Unloaded NP		0
Hydrocortisone polybutylcyanoacrylate nanoparticles		
Adsorptive drug loading		
1AZ/1NP	162.5 ± 10.6	57.9 ± 4.3
0.5AZ/1NP	155 ± 7.1	65.1 ± 4.2
0.1AZ/1NP	145 ± 7.1	69.5 ± 4.7
Unloaded NP	115 ± 21.2	0
Doxorubicin polybutylcyanoacrylate nanoparticles		
Incorporative drug loading		
1AZ/1NP	257.5 ± 10.6	72.35 ± 5.6
0.5AZ/1NP	247.5 ± 10.6	86.5 ± 5.8
0.1AZ/1NP	220 ± 14.14	94.4 ± 5.9
Unloaded NP	160 ± 14.14	0
Dalargin polybutylcyanoacrylate nanoparticles		
Adsorptive drug loading		
1AZ/1NP	227.5 ± 10.6	27.7 ± 3.4
0.5AZ/1NP	217.5 ± 10.6	28.5 ± 3.4
0.1AZ/1NP	190 ± 14.14	31.8 ± 3.2
Unloaded NP	115 ± 21.21	0

[a] 1AZ/1NP represents the stock drug nanoparticle preparations, 0.5AZ/1NP a 1:2 dilution of the drug at the same nanoparticle concentration, 0.1AZ/1NP the 1:10 drug dilution (see Section 2).

butylcyanoacrylate nanoparticles was about 100–130 nm. The diameters of the drug nanoparticles preparations are shown in Table 1. Incorporative drug loading of nanoparticles yielded larger diameters than adsorptive loading. The diameter increased with higher drug contents of nanoparticles with the same drug. With methylene blue nanoparticles no particle size determination was

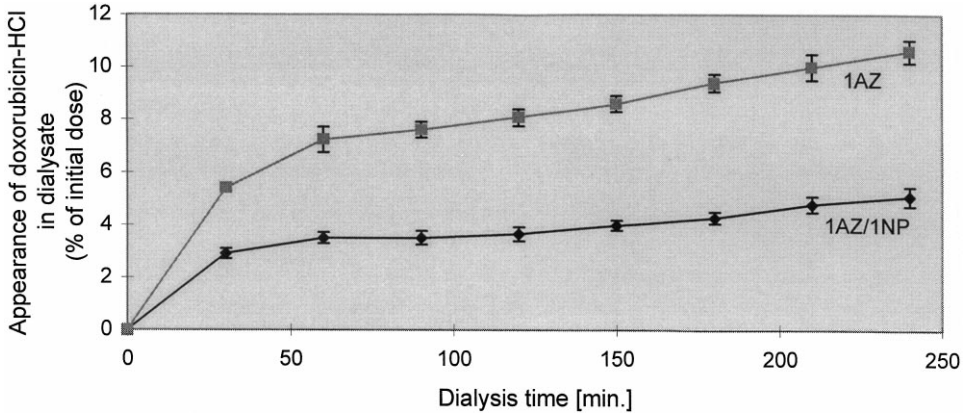

Fig. 1. Dialysis of doxorubicin-HCl in free solution (1AZ) or incorporated within polybutylcyanoacrylate nanoparticles (1AZ/1NP) (membrane: cellulose acetate, $n = 6$). 1AZ refers to the stock drug solution, 1AZ/1NP to the respective drug nanoparticle preparation (see Section 2). 1AZ/1NP–1AZ: the experiment started with 9.15 mg doxorubicin–HCl in each donor-compartment (7 ml 1AZ/1NP-particle suspension or 6.86 ml 1AZ - particle-free solution).

possible by photon correlation spectroscopy since its colour interfered with the laser colour.

3.2. Drug loading

The drug loading of the nanoparticles is also shown in Table 1 and depends on the drug characteristics as well as on its concentration. In addition, the type of drug loading—incorporative or adsorptive—is important. [^{75}Se]norcholestenol, captopril, methylene blue, and doxorubicin nanoparticles were produced by the incorporative drug loading technique, and captopril, hydrocortisone, and dalargin nanoparticles by adsorption to empty nanoparticles. Incorporative drug loading to nanoparticles yielded a higher drug association than the adsorption. Drug loading to polybutylcyanoacrylate nanoparticles ranged from over 97% with captopril produced by incorporation to 27.7% with dalargin produced by adsorption. With the exception of the self associating methylene blue relative drug loading becomes higher if the drug to nanoparticle ratio is smaller. Dilution of drug–nanoparticle-suspensions decreases the amount of nanoparticle bound drug because of desorption of the drug and a resulting shift of the adsorption equilibrium between nanoparticle associated and free drug, and, hence, led to smaller drug loading.

3.3. Dialysis experiments

A typical example of a dialysis experiment with one of the drugs—doxorubicin—appearing in the receiver chamber is shown in Fig. 1. After about 70 min the drug appearance curves in the receiver chamber became linear and the permeabilities could be calculated and are listed in Table 2. Captopril bound to nanoparticles by incorporation was not released and, hence, not transported and therefore is not shown. The statistical evaluation is shown in Table 3.

The permeabilities varied with the type of drug, the drug and nanoparticle concentration, and the type of membrane. Large differences were observed between drugs used at the same initial concentration in the donor cells, i.e. doxorubicin, dalargin, and hydrocortisone solution and nanoparticles, demonstrating the importance of the physico chemical properties of the drugs which in turn govern the membrane–drug interactions, Table 2. The molecular weights of these drugs would have suggested the opposite rank order.

With the exception of captopril the permeabilities of the drug solutions increased with decreasing concentrations (except dalargin and [^{75}Se]norcholestenol, pig small gut 0.5AZ versus 0.1AZ = n.s.) indicating a mutual impediment of

Table 2
Permeability coefficients (P [cm/s] 11×0^{-6}) ± S.D. of different drug preparations and concentrations ($n = 6$). Drug was associated with polybutylcyanoacrylate nanoparticles (AZ/NP) or in equimolar solution (AZ)[a]

Membrane	Drug-NP concentration							
	1AZ/1NP	0.5AZ/0.5NP	0.1AZ/0.1NP	0.5AZ/1NP	0.1AZ/1NP	1AZ	0.5AZ	0.1AZ
[^{75}Se]norcholestenol								
Cellulose acetate	7.33 ± 0.87	10.05 ± 0.75	11.13 ± 1.24	11.4 ± 0.64	13.05 ± 0.97	2.05 ± 0.37	3.95 ± 0.59	5.05 ± 0.48
Pig small gut	3.13 ± 1.35	3.75 ± 1.14	3.98 ± 1.18	3.95 ± 1.35	4.65 ± 1.44	1.7 ± 0.93	2.63 ± 1.2	1.55 ± 0.96
Silicon rubber	1.71 ± 0.24	2.48 ± 0.49	3.1 ± 0.42	3.48 ± 0.51	3.73 ± 0.43	0.345 ± 0.18	1.23 ± 0.29	1.45 ± 0.24
Hairless mice skin	0.65 ± 0.26	0.8 ± 0.29	1.1 ± 0.48	1.05 ± 0.37	1.5 ± 0.56	0.2 ± 0.08	0.33 ± 0.17	0.7 ± 0.32
Captopril								
Cellulose acetate	7.13 ± 0.51	3.95 ± 0.42	2.65 ± 0.37	2.98 ± 0.35	2.25 ± 0.34	53.08 ± 3.14	44.43 ± 3.22	40.28 ± 1.66
Pig small gut	2.23 ± 0.83	1.58 ± 0.8	1.23 ± 0.39	1.1 ± 0.37	0.83 ± 0.38	5.58 ± 0.74	4.15 ± 0.73	3.15 ± 0.8
Silicon rubber	1.23 ± 0.21	0.93 ± 0.19	0.63 ± 0.17	0.5 ± 0.08	0.375 ± 0.15	3.73 ± 0.3	3.05 ± 0.37	2.1 ± 0.26
Hairless mice skin	1.0 ± 0.25	0.7 ± 0.16	0.45 ± 0.21	0.4 ± 0.16	0.3 ± 0.16	3.25 ± 0.42	2.85 ± 0.34	1.35 ± 0.17
Hydrocortisone								
Cellulose acetate	4.48 ± 0.68	6.1 ± 0.71	7.45 ± 0.95	3.73 ± 0.81	3.03 ± 0.56	15.1 ± 1.6	23.35 ± 2.16	36.33 ± 2.99
Methylene blue								
Cellulose acetate	3.1 ± 0.62	3.83 ± 0.59	4.15 ± 0.7	28.35 ± 1.71	38.3 ± 2.86	82.3 ± 9.6	85.95 ± 8.3	94.08 ± 10.03
Doxorubicin								
Cellulose acetate	11.23 ± 3.61	56.08 ± 6.74	90.75 ± 7.37	9.25 ± 1.36	5.15 ± 0.72	28.5 ± 6.24	161.65 ± 10.71	187.3 ± 22.21
Dalargin								
Cellulose acetate	27.98 ± 2.83	58.3 ± 6.44	49.37 ± 6.26	56.47 ± 7.9	41.13 ± 5.7	36.1 ± 5.96	72.3 ± 7.24	69.88 ± 7.98

[a] 1AZ/1NP represents the stock drug/nanoparticle preparation, 0.5 the respective 1:2 and 0.1 the respective 1:10 dilutions (see Section 2).

Table 3
Statistical evaluation of the differences in permeability calculated by Student's *t*-test[a]

Membrane	Drug-NP-concentration								
	1AZ/1NP – 0.5AZ/0.5NP	0.5AZ/0.5NP – 0.1AZ/0.1NP	1AZ/1NP – 0.1AZ/0.1NP	0.5AZ/1NP – 0.5AZ/0.5NP	0.1AZ/1NP – 0.1AZ/0.1NP	1AZ/1NP – 1AZ	1AZ – 0.5AZ	0.5AZ – 0.1AZ	1AZ – 0.1AZ
[^{75}Se]norcholestenol									
Cellulose acetate	<0.005	n.s.	<0.005	<0.05	<0.05	<0.001	<0.005	<0.025	<0.001
Pig small gut	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Silicone rubber	<0.05	n.s.	<0.005	<0.05	n.s.	<0.001	<0.005	n.s.	<0.001
Hairless mice skin	n.s.	n.s.	n.s.	n.s.	n.s.	<0.02	n.s.	n.s.	<0.025
Captopril									
Cellulose acetate	< 0.001	< 0.005	< 0.001	<0.02	n.s.	<0.001	<0.01	<0.05	<0.001
Pig small gut	n.s.	n.s.	n.s.	n.s.	n.s.	<0.005	<0.05	n.s.	<0.01
Silicone rubber	n.s.	<0.05	<0.01	<0.01	n.s.	<0.001	<0.025	<0.01	<0.001
Hairless mice skin	n.s.	n.s.	<0.02	<0.05	n.s.	<0.001	n.s.	<0.001	<0.001
Hydrocortisone									
Cellulose acetate	<0.02	<0.05	<0.005	<0.01	<0.001	<0.001	<0.005	<0.001	<0.001
Methylene blue									
Cellulose acetate	n.s.	n.s.	n.s.	<0.001	<0.001	<0.001	n.s.	n.s.	n.s.
Doxorubicin									
Cellulose acetate	<0.001	<0.005	<0.001	<0.001	<0.001	<0.005	<0.001	n.s.	<0.001
Dalargin									
Cellulose acetate	<0.001	n.s.	<0.005	n.s.	n.s.	<0.05	<0.001	n.s.	<0.001

[a] N.s., non significant. Symbols, 1AZ/1NP, etc. are explained in Table 2 and in more detail in Section 2.

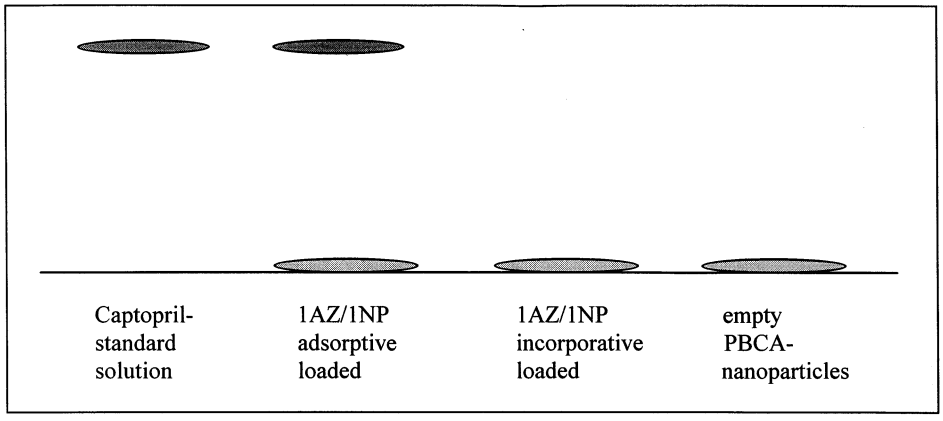

Fig. 2. Thin layer chromatogram of captopril nanoparticles.

the drug molecules during their membrane diffusion at higher concentrations. In contrast, captopril shows a higher permeability at higher concentrations. This is probably caused by a high binding of this drug to the membranes which is more relevant for the permeability at lower drug concentrations than at higher ones. This can be substantiated by the fact that this drug also binds extremely well to other materials, as for instance nanoparticles.

With another exception, in this case [^{75}Se]norcholestenol, free drugs yielded much higher permeabilities than nanoparticle associated drugs. This shows that these drugs are released into the medium prior to transport in free form through the membrane and that—as expected—nanoparticles act as a slow release system. This situation is different with [^{75}Se]norcholestenol. This drug is very insoluble and, therefore, was provided in a micellar polysorbate 80 solution. Surfactants such as polysorbates can cause considerable interfacial barriers, retarding the transport of solutes solubilized in the micelles by orders of magnitude (Bikhazi and Higuchi, 1970, 1971; Surpuriya and Higuchi, 1972a,b). As a consequence, nanoparticles seem to enable a much more direct delivery to the membrane and consequently a much higher transport rate, as already suggested by Kreuter et al. (1983). This is also substantiated by the fact that by increasing the nanoparticle to drug ratio (0.5AZ/0.5NP versus 0.5AZ/1NP and 0.1AZ/0.1NP versus 0.1AZ/1NP) the permeabilities of [^{75}Se]norcholestenol were increased, whereas with all the other drugs except methylene blue the opposite was seen. Methylene blue also is an exception to this rule due to its tendency to self associate. This abnormality of methylene blue is also reflected by the above mentioned fact that methylene blue binds to nanoparticles better at higher loading concentrations and at higher drug to nanoparticle ratios.

Captopril not only shows a reduced permeability of the drug at higher concentrations in free form but also after binding to nanoparticles. It is not liberated after polymerization, i.e. incorporation, in the presence of the drug, and, consequently, no membrane transport occurred (data not shown). Also the thin layer chromatogram shows no drug liberation after incorporation but a significant liberation after adsorption (Fig. 2). All other drugs exhibited lower permeabilities with higher drug and nanoparticle concentrations (except dalargin 0.5AZ/0.5NP = n.s.) and except for the above discussed special cases of [^{75}Se]norcholestenol and methylene blue with a higher nanoparticle to drug ratio. These lower permeabilities are a reflection of the reduced drug liberation rate at lower drug concentrations and drug to nanoparticle ratios.

[^{75}Se]norcholestenol is a γ emitter which enables the easy establishment of the mass balance after termination of the dialysis experiment. Therefore, the amounts of loosely adsorbed drug removable with a Kleenex® tissue (Kimberly Clark, Koblenz/Rheinhafen, Germany) and tightly adsorbed drug removable with an adhesive tape (Tesafilm®,

Beiersdorf, Hamburg, Germany) were also determined. The resulting concentration profile after 5 h of dialysis showed that the highest dose of [^{75}Se]norcholestenol remained in the donor chamber (about 64%), about 19% was tightly adsorbed on the membrane, about 16% arrived in the receiver chamber, about 1% was loosely adsorbed on the membrane, and about 0,1% was entrapped into the membrane. The high amount that is tightly bound to the membrane shows the bioadhesive properties of nanoparticles that were suggested previously (Diepold et al., 1989a,b).

Among the different membranes the cellulose acetate membrane yielded the highest permeabilities followed by pig small intestine, silicone, and finally hairless mouse skin. The trends for the different preparations of the two drugs employed with all membranes, [^{75}Se]norcholestenol and captopril, remained the same as already discussed above. The ratios between the different permeability coefficients remained proportional. Interestingly, but not unexpectedly, the S.D. for hairless mice skin and especially pig small intestine were much larger than for the artificial membranes and in most cases yielded statistically non significant differences.

The results of the above in vitro dialysis experiments demonstrate that nanoparticles efficiently modify the absorption of drugs. In most cases association to polybutylcyanoacrylate nanoparticles gains sustained controlled drug release.

4. Conclusions

These results indicate that the enhanced drug delivery observed with [^{75}Se]norcholestenol represents a unique phenomenon that probably was due to the presence of polysorbate 80 in the control solution. The formation of micelles by this surfactant seems to impede the interaction of the drug with the membrane surface similarly to that observed with liposomes (Kreuter et al., 1981) and consequently reduces the transport rates.

An enhanced transport of nanoparticle-bound captopril as reported by Scherer (1992) was not observed in the present study. No explanation could be found for this discrepancy. Likewise with all other drugs investigated here no enhanced drug transport across any of the membranes used in this study was observed.

It can be concluded that nanoparticles in most cases do not enhance but, in contrast, retard the delivery of drugs across natural and artificial membranes. With very lipophilic drugs such as [^{75}Se]norcholestenol, however, the retarding effect may be lower than that of micelles.

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