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Chitosan solutions and particles: Evaluation of their permeation enhancing potential on MDCK cells used as blood brain barrier model

Juliane Hombach, Andreas Bernkop-Schnürch*

Institute of Pharmacy, Leopold-Franzens-University Innsbruck, Innrain 52, Josef-Möller-Haus, 6020 Innsbruck, Austria

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ABSTRACT

It was the aim of the present study to investigate the potential of chitosan of different molecular weight in solution and as particles to enhance the transport into the brain. FITC-dextran 4 (FD4) transport with and without chitosans of different molecular weight across MDCK cell monolayers, a model for the blood brain barrier, was compared. In the following particles of chitosan exhibiting the most appropriate molecular weight were prepared and their particle size and stability were evaluated. Furthermore permeation studies, MDCK cell toxicity test and red blood cell lysis test were performed. The rank order for chitosan for permeation enhancement across MDCK cells was determined to be 20 kDa ~ 150 kDa > 400 kDa ~ 600 kDa. Moreover particles showed a higher permeation enhancement than the corresponding solution and the smaller the particles were the higher the permeation of FD4 was. All particles were stable for 72 h. Particles displayed increased MDCK cell toxicity and red blood cell lysis compared to chitosan in solution. The smaller the particles were, the higher their toxicity was. According to these results chitosan particles are more potent in absorption enhancement than chitosan solutions.

1. Introduction

The blood brain barrier (BBB) is an important very tight barrier to protect the brain from penetration of substances. A lot of drugs such as antibiotics, virustatics and cytostatics achieve only low concentrations at their site of action when administered parenterally and therefore have to be applied directly into the brain. The most acceptable in vitro model for the blood brain barrier is currently the primary bovine brain endothelial cells (Gumbleton and Audus, 2001). However, it is generally difficult to establish and maintain primary cultures, time and labour intensive to isolate the cells, batches can vary a lot and animals are needed each time cultures are established. Immortalised brain capillary endothelial cell lines such as bEND5 or RBE4 are closer to the in vivo cell phenotype but fail to generate a restrictive paracellular barrier (Gumbleton and Audus, 2001). A continuous cell line is easier to handle. MDCK (Madin-Darby canine kidney) cells are a possible alternative model to mimic the transport across the blood brain barrier, as they express P-glycoprotein and also express the tight junction proteins Claudin-1, -4 and occludine (Sjo et al., 2003), which are important to form a restrictive paracellular barrier with tight junctions. Furthermore Garberg et al. (2005) found the highest in vitro in vivo correlation in the MDCKwt cell model when various compounds being transported by passive diffusion or by carrier mediated influx or being actively excreted by efflux were analysed. The correlation was even better when only the compounds crossing the BBB by passive diffusion were analysed. Therefore MDCK cells were chosen as a cell model for permeation experiments utilizing the paracellularly absorbed fluorescence labelled dextran 4 kDa (FD4) as model compound.

Because of their small size nanoscale materials have the potential to cross the blood brain barrier and open a new way for brain drug delivery (de Jong and Borm, 2008). Different types of chemical structures and materials, e.g. polymers, dendrimers, fullerenes or natural materials, can be used to prepare nanocarriers for drug delivery (Borm and Muller-Schulte, 2006).

Chitosan is a natural-origin cationic polysaccharide, which is non-toxic even after intravenous application (Rao and Sharma, 1997), biocompatible, slowly biodegradable after oral administration and soluble at pH values below 6.5. Besides its mucoadhesive and controlled release properties chitosan is also able to increase the paracellular permeability due to the opening of tight junctions which has been shown for various routes of delivery such as for nasal (Illum et al., 1994) or intestinal drug delivery (Artursson et al., 1994). However, no effect on the paracellular permeability could be observed at pH 7.4 (Borchard et al., 1996), which is the physiological pH of blood. This indicates that chitosan solutions are not effective as permeation enhancer at neutral pH values, because of the missing solubility of chitosan at neutral and alkaline pH values. Chitosan in form of particles can overcome this problem, because particles need not to be dissolved. Chitosan nanoparticles are widely used as

* Corresponding author. Tel.: +43 512 507 5383; fax: +43 512 507 2933.
E-mail address: andreas.bernkop@uibk.ac.at (A. Bernkop-Schnürch).

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nonviral carriers for plasmids, proteins, peptides, oligonucleotides and other drugs. Chitosan nanoparticles can protect incorporated drugs from enzymatic degradation (Mao et al., 2001) and enhance the intestinal absorption in vivo in rats (Pan et al., 2002).

It was the aim of this study to produce and characterize chitosan particles and investigate their permeation enhancing properties across MDCK cells. Furthermore, toxicity aspects will be assessed.

2. Materials and methods

2.1. Materials

Chitosan low-viscous (LV) and chitosan highly viscous (HV) were obtained from Fluka (Vienna, Austria), chitosan low molecular weight (LMW), chitosan medium molecular weight (MMW) and chitosan high molecular weight (HMW) from Sigma–Aldrich (Vienna, Austria) and D-glucosamine hydrochloride from Acros Organics (Geel, Belgium) as specified in Table 1. Chitosan ultra low molecular weight (ULMW) was prepared by treating chitosan medium molecular weight with sodium nitrite as described by Schmitz et al. (2007). Hank's balanced salts for Hank's balanced salts solution (HBSS), 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), sodium triphosphate pentabasic (TPP), cimetidine, propranolol and rhodamine 123 (rho 123) were purchased from Sigma–Aldrich (Vienna, Austria). Fluoresceinisothiocyanate-dextran 4 (FD4) was obtained from TdB Consultancy AB (Uppsala, Sweden). MDCK cells wild type were kindly donated by Prof. Pfaller, Institute of Physiology, Medical University of Innsbruck. Cell culture medium was prepared by using Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin solution (100 U penicillin and 0.1 mg of streptomycin per litre medium) (all substances were purchased from Sigma–Aldrich, Vienna, Austria) and 10% fetal calf serum (FCS) (Gibco, Carlsbad, CA, USA). Corning costar© transwell© with polycarbonate membrane, 12 mm diameter, 0.4 μm pore size, clear polyester membranes were obtained from Corning (Acton, MA, USA).

All other reagents were of analytical grade and received from commercial sources.

2.2. Methods

2.2.1. Particle preparation and characterisation

In order to generate chitosan particles with different sizes one single method would have been preferred. However three different methods had to be used, as no single method was able to form particles of so different sizes. The particle sizes result out of each method used. There were no surfactants or dispersing agents except water added, in order to exclude any damaging effect of these auxiliary agents on the cell membrane.

2.2.1.1. Ultraturax. 0.3 g of chitosan LV was suspended in 100 ml of demineralised water using an Ultraturax mixer (EutoTurax T20b, IKA Labortechnik, Staufen, Germany) with 20,000 rpm for 10 min.

2.2.1.2. High pressure homogenizer. 80 ml of a 0.3% (m/v) chitosan LV in distilled water suspension was prehomogenized with three cycles at 500 bar and further homogenized with 10 cycles at 1500 bar by a high pressure homogenizer (MiniDeBEE, HIP, Erie, PA).

2.2.1.3. Ionic gelation. Cationic chitosan is able to form particles with negatively charged TPP by ionic gelation. Therefore, 6 mg of chitosan LV were dissolved in 3 ml of water pH 6. A 0.2% (m/v) TPP solution was slowly dropwisely added until turbidity occurred. The pH was finally adjusted to 7.4 with NaOH.

2.2.1.4. Particle size determination. The particle size of particles smaller than 1 μm was determined with a Nicomp particle sizer (Nicomp 380 ZLS, PSS Nicomp, Santa Barbara, CA) by dynamic light scattering. In contrast, particle size measurements for particles larger than 1 μm were performed on a laser diffraction particles size analyser (analysette 22 compact version, Fritsch GmbH, Idar Oberstein, Germany). Particle sizes were determined directly after preparation, after 24 h and after 72 h, respectively, in order to investigate the stability of particles. In the meantime particle solutions were stored at room temperature.

2.2.2. Permeation across MDCK monolayers

2.2.2.1. MDCK cell maintenance. MDCK cells were maintained in the medium described above at 95% humidity and 37 °C in an atmosphere of 5% CO_2 in 75 cm^2 plastic flasks (Corning Costar, Szabo Scandic, Vienna, Austria). Medium was changed three times a week and cells were splitted once a week.

The following experiments were performed with cells of passage 77–96. Cells were plated in a density of 1×10^6 cells onto the polycarbonate membrane inserts of 12-well plates directly after splitting and were allowed to grow and to differentiate for 4–7 days for permeation experiments. During this time the cells were fed with 1 ml of the medium mentioned above pH 7.4 in the apical and 2 ml of the same medium in the basolateral chamber every 48 h.

For cytotoxicity studies cells were seeded on 12-well microtiter plates in a density of 1×10^6 cells per well and incubated for 24 h before the experiment.

2.2.2.2. Permeation with chitosan of different molecular weight and chitosan particles of different size. Permeation studies were performed in the transwell monolayer system with a volume of 1 ml of HBSS buffered with 40 mM Hepes pH 6.4 in the donor and the same medium pH 7.4 in the acceptor chamber, a permeation area of 1.13 cm^2, at 37 °C in an atmosphere of 5% CO_2 and 95% humidity. The absorptive transport of FD4 in a final concentration of 1 mg/ml was investigated in the absence and presence of glucosamine, chitosan ULMW, chitosan LV, chitosan LMW, chitosan MMW, chitosan HV and chitosan HMW applied in a concentration of 0.1% (m/v) and 0.25% (m/v), respectively, in order to identify the most suitable molecular weight.

Furthermore chitosan particles of different size applied in a concentration of 0.1% (m/v) were tested for permeation enhancement and compared with chitosan in solution. To the sample of particles

Table 1
Characterisation of the used chitosans.

Name	Source	Molecular weight	Viscosity (mPa s)	Deacetylation degree
(1) D-Glucosamine hydrochloride	Acros Organics	0.2 kDa	Not determined	No acetylation
(2) Ultra low molecular weight (ULMW)	Self-prepared	10–20 kDa	Not determined	75–85%
(3) Low-viscous (LV)	Fluka	~150 kDa	<200	95–98%
(4) Low molecular weight (LMW)	Sigma	~150 kDa	20	75–85%
(5) Medium molecular weight (MMW)	Sigma	~400 kDa	200	75–85%
(6) Highly viscous (HV)	Fluka	~600 kDa	>400	75–85%
(7) High molecular weight (HMW)	Sigma	~600 kDa	800	>75%

produced by Ultraturax and high pressure homogenizer TPP was additionally added to exclude the permeation enhancing effect of TPP. For permeation studies with particles in both donor and acceptor chamber HBSS buffered with 40 mM Hepes pH 7.4 was used.

MDCK cell monolayer integrity was controlled by permeation of low permeable cimetidine and rho 123 and high permeable propranolol.

Within 3 h incubation time samples of 100 μl were taken out of the acceptor chamber and substituted by incubation medium preheated at 37 °C every 30 min. The amount of permeated FD4 was determined by fluorescence measurements (λ_{ex} = 485 nm and λ_{em} = 535 nm) by a microplate reader (Tecan Austria GmbH, Grödig, Austria). Cumulative corrections were made for previously removed samples.

2.2.2.3. TEER measurement. Transepithelial electrical resistance (TEER) of the monolayers was measured with the EVOM instrument (World Precision Instruments, Sarasota, FL). The TEER of untreated cells and cells treated with chitosan solution and different particles was determined in DMEM before the experiment, after the experiment and after 24 h. The measured TEER before the experiment was set as 100% and all other values were calculated according to this.

2.2.3. Toxicity studies

2.2.3.1. Red blood cell (RBC) lysis. Erythrocytes (blood group A1B, RhD positive) were diluted with red blood cell lysis buffer (PBS) to a final concentration of 6×10^8 cells/ml. 100 μl were transferred to each well of a 96-well microtitration plate. 100 μl of a chitosan LV solution and chitosan particles of different sizes were added in different concentrations to the wells. Red blood cell lysis buffer was used as negative control and 2% (m/v) Triton® X 100 as positive control causing 100% hemoglobin release. The experiment was performed four times for each sample. After 1 h incubation time the microtitration plate was centrifuged at 1000 × *g* for 10 min and the supernatants (100 μl) was transferred into another microtitration plate. Hemoglobin release was determined photometrically at 570 nm with a microplate reader (Tecan Austria GmbH, Grödig, Austria).

2.2.3.2. LDH assay. To evaluate the MDCK cell toxicity of the different particles, a colorimetric assay for the quantification of cell death and cell lysis, based on the measurement of lactate dehydrogenase (LDH) activity released from the cytosol of damaged cells into the supernatant was used (Decker and Lohmannmatthes, 1988; Legrand et al., 1992). Cells were exposed over a time period of 3 h to chitosan LV solution and chitosan LV particles of different size in a concentration of 0.1% (m/v) in HBSS pH 6.4 for the solution and 7.4 for the particles. Particles having been prepared by Ultraturax and high pressure homogenizer were tested with and without added TPP to exclude TPP toxicity. As negative control HBSS pH 6.4 and 7.4 and as positive control 2% (m/v) Triton® X 100 in HBSS was used.

Supernatant was withdrawn at time points 0 min and 180 min, samples were centrifuged at 250 × *g* for 4 min and stored at 4 °C before analysis using the cytotoxicity detection kit by Roche (Basel, Switzerland). Absorbance was measured after 15 min incubation time in the dark photometrically at a wavelength of 492 nm using a microplate reader (Tecan Austria GmbH, Grödig, Austria).

2.2.4. Data analysis and statistics

Apparent permeability coefficients (P_{app}) for FD4 were calculated as follows:

$$P_{app} = \frac{Q}{A \times c \times t} \quad (1)$$

where Q is the total amount permeated within 3 h (μg), A is the diffusion area of the Ussing-type chambers (0.64 cm^2) or the transwell (1.13 cm^2), respectively, c is the initial concentration in the donor chamber (1000 μg/cm^3), and t is the time of the experiment (10,800 s). Improvement ratios were calculated from P_{app} values for each test compound by:

$$\text{improvement ratio} = \frac{P_{app}(\text{FD4} + \text{chitosan solution})}{P_{app}(\text{FD4 only})} \quad (2)$$

Results of the RBC lysis test were calculated as follows:

$$\text{RBC lysis}\,(\%) = \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{buffer}})}{(\text{Abs}_{\text{Triton}} - \text{Abs}_{\text{buffer}})} \times 100 \quad (3)$$

The percentage of cell toxicity in the LDH assay was calculated by:

$$\text{cytotoxicity}\,(\%) = \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{HBSS}})}{(\text{Abs}_{\text{Triton}} - \text{Abs}_{\text{HBSS}})} \times 100 \quad (4)$$

Statistical data analyses were performed using Student's *t*-test with $p < 0.05$ as the minimal level of significance. Calculations were done using the software Analyse-it for Microsoft Excel.

3. Results and discussion

The suitability of the MDCK monolayers was tested with low permeable FD4, rho 123, cimetidine and high permeable propranolol. The determined P_{app} value of FD4 of 0.24×10^{-6} cm/s is in the range of 0.12×10^{-7} cm/s (Furuse et al., 2001) to 1.1×10^{-6} cm/s (McCarthy et al., 2000) found in the literature. The low permeating drugs cimetidine and rho 123 showed only low permeation and high permeating control propranolol demonstrated almost 100-fold higher permeation. Moreover, the P_{app} values of the other markers and the initial TEER value are also close to those found in literature (cimetidine and TEER (Garberg et al., 2005), rho 123 (Wang et al., 2005)).

The results of the permeation studies with chitosans of different molecular weight are listed in Table 2. Generally, permeation of FD4 in combination with 0.25% (m/v) chitosans was more improved than with 0.1% (m/v) chitosans. For example chitosan LV is able to enhance paracellular FD4 transport almost 13-fold in a concentration of 0.1% (m/v) and even more than 20-fold in a concentration of 0.25% (m/v). The rank order of chitosan for paracellular permeation enhancement across MDCK cells was found to be chitosan LV > chitosan LMW ~ chitosan ULMW > chitosan MMW > glucosamine > chitosan HV ~ chitosan HMW. Both chitosan with higher molecular weight (400–600 kDa) and glucosamine (the chitosan monomer) have a less permeation enhancing effect than chitosan with a low molecular weight (20–150 kDa). A high molecular weight is not advantageous as long polymer chains are less flexible and seem to reach the tight junction proteins more difficultly. Shah et al. (2008) increased the P_{app} of acyclovir and ^{99m}Tc-mannitol with 0.1% (m/v) and 0.3% (m/v) chitosan (20 kDa) solutions approximately 7-fold and 10-fold, respectively, which is similar to our results. The mechanism of chitosan mediated disruption of tight junctions is the translocation of tight junction proteins from the membrane to the cytoskeleton (Smith et al., 2004).

The highest permeation enhancement was found for chitosan LV (~150 kDa) solutions. Both higher and lower molecular weight chitosans showed less permeation enhancement. Therefore chitosan LV was chosen to prepare particles. With three different methods particles of three different size ranges could be produced. The smallest size of approximately 300 nm was achieved by ionic gelation. After homogenisation particles were around 900 nm in size and particles having been prepared by Ultraturax had a mean size of 12,000 nm. The exact size distribution is shown in Fig. 1. In Table 3 it is demonstrated that the size of all three particle types, when stored at room temperature, does not change significantly within 72 h, which means that stable particles have been produced. The

Table 2
Comparison of the P_{app} values of several control solutions and comparison of the P_{app} values and improvement ratios of 0.1% (m/v) and 0.25% (m/v) solutions of chitosan with different molecular mass, respectively. Indicated values are means ± SD ($n = 3$).

	P_{app} [$\times 10^{-6}$ cm/s]			
FD4	0.24 ± 0.05			
Cimetidine	0.37 ± 0.05			
rho 123	0.65 ± 0.08			
Propranolol	24.5 ± 5.1			
	0.1% (m/v)		0.25% (m/v)	
	P_{app} [$\times 10^{-6}$ cm/s]	Improvement ratio	P_{app} [$\times 10^{-6}$ cm/s]	Improvement ratio
FD4 + glucosamine	n.d.	n.d.	0.68 ± 0.05*	2.8
FD4 + chitosan ULMW	1.37 ± 0.07*	5.7	3.79 ± 0.27*	15.8
FD4 + chitosan LV	3.02 ± 0.28*	12.6	4.86 ± 0.80*	20.3
FD4 + chitosan LMW	1.44 ± 0.33*	6.0	2.86 ± 0.46*	11.9
FD4 + chitosan MMW	0.32 ± 0.03	1.3	1.51 ± 0.14*	6.3
FD4 + chitosan HV	n.d.	n.d.	0.58 ± 0.14*	2.4
FD4 + chitosan HMW	0.33 ± 0.04	1.4	0.51 ± 0.03*	2.1

n.d.: not determined.
* Differs from FD4, $p < 0.05$.

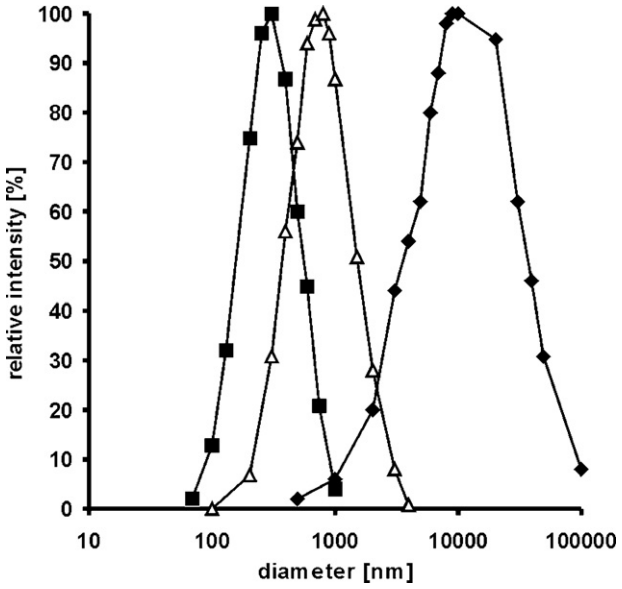

Fig. 1. Particle size distribution of the used chitosan particles prepared by Ultraturax 12,000 nm U (♦), Homogenizer 900 nm H (△) and via ionic gelation 300 nm G (■).

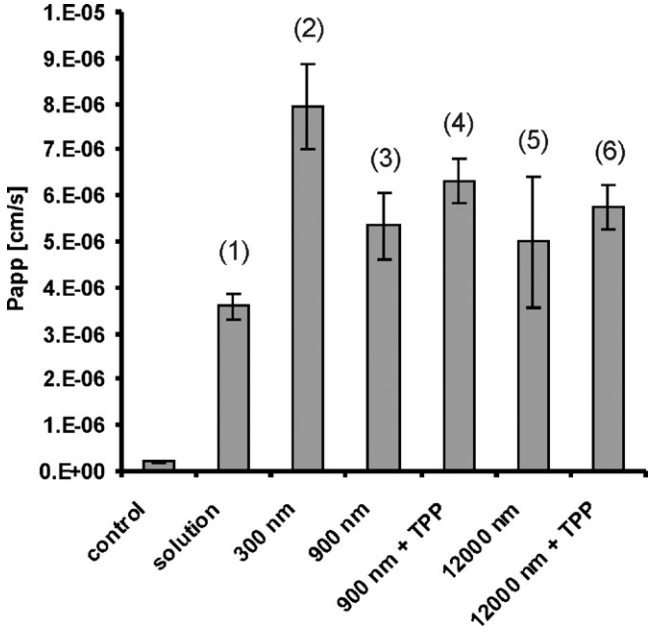

Fig. 2. Comparison of P_{app} values of chitosan LV in solution and particles of indicated size. Values are means ± SD of at least three experiments. (1) Differs from control ($p < 0.00001$). (2) Differs from control and solution ($p < 0.00001$). (3) Differs from control, solution and 300 nm ($p < 0.00001$). (4) Differs from control, solution ($p < 0.00001$) and 300 nm ($p < 0.006$), does not differ from 900 nm ($p > 0.05$). (5) Differs from control ($p < 0.0005$), solution ($p < 0.03$) and 300 nm ($p < 0.001$), does not differ from 900 nm ($p > 0.30$) and 900 nm + TPP ($p > 0.09$). (6) Differs from control ($p < 0.003$), solution ($p < 0.005$) and 300 nm ($p < 0.0008$), does not differ from 900 nm ($p > 0.16$), 900 nm + TPP ($p > 0.11$) and 12,000 nm ($p > 0.14$).

polydispersity index (PI) is a parameter for particle size distribution. A PI lower than 0.5 as it is the case for all particles prepared via ionic gelation and by homogenization indicates a small discrete size distribution. Beneficial for particles is, that particle suspensions can be used at physiological pH 7, whereas chitosan is not soluble at this pH.

Permeation studies across MDCK monolayers were also performed with these particles and the resulting P_{app} values of FD4 are shown in Fig. 2. All particles increased FD4 transport even more than chitosan LV solution, whereas the smallest particles of 300 nm showed the most pronounced effect. To particles, which were not prepared by ionic gelation, TPP was additionally added to exclude the influence of TPP on permeation enhancement. Particles with

Table 3
Particle size of chitosan LV particles produced by different methods and its polydispersity indices (PI) having been determined directly after production and after 24 h and 72 h storage at room temperature ($n = 3$).

	After production		After 24 h		After 72 h	
	Size [nm]	PI	Size [nm]	PI	Size [nm]	PI
Ultraturax (12,000 nm U)	12,297 ± 4,708	n.d.	10,336 ± 3,215*	n.d.	16,224 ± 5,841*	n.d.
Homogenizer (900 nm H)	873 ± 464	0.28	899 ± 535*	0.35	850 ± 300*	0.21
Ionic gelation (300 nm G)	324 ± 205	0.40	298 ± 162*	0.30	280 ± 161*	0.33

n.d.: not determined.
* Does not differ from size after production ($p > 0.05$).

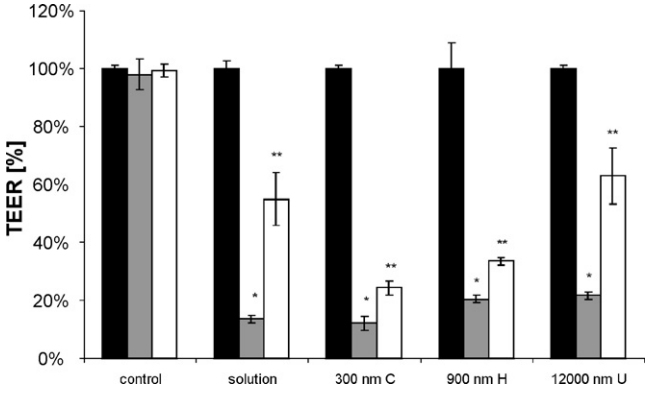

Fig. 3. Transepithelial electrical resistance before permeation (black bars), after permeation (grey bars) and after 24 h (white bars) of FD4 as control only and in the presence of 0.1% (m/v) of chitosan LV solution, 300 nm G particles, 900 nm H particles and 12,000 nm U particles, respectively, across MDCK monolayers. Data are means ± SD of three experiments. *Differs from value before permeation ($p < 0.05$). **Differs from value before and after permeation ($p < 0.05$).

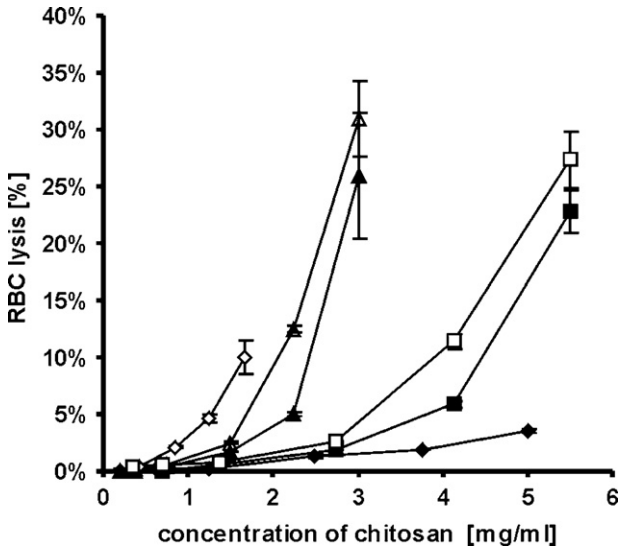

Fig. 4. RBC lysis [%] after incubation for 1 h with chitosan LV solution (♦), 300 nm G particles (◊), 900 nm H particles without (▲) and with (△) additional TPP and 12,000 nm U particles without (■) and with (□) additional TPP in different concentrations. Indicated values are means ± SD of four experiments.

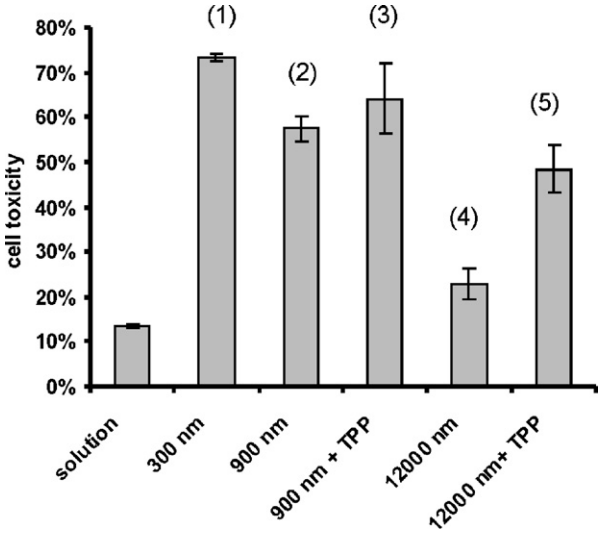

Fig. 5. Cell toxicity of 0.1% (m/v) of indicated test compounds determined by LDH test on MDCK cells for 3 h. Indicated values are means ± SD of three experiments. (1) Differs from solution ($p < 0.0003$). (2) Differs from solution ($p < 0.0006$) and 300 nm ($p < 0.004$). (3) Differs from solution ($p < 0.04$), does not differ from 300 nm ($p > 0.18$) and 900 nm ($p > 0.23$). (1) Differs from solution ($p < 0.04$), 300 nm ($p < 0.001$), 900 nm ($p < 0.0005$) and 900 nm + TPP ($p < 0.03$). (5) Differs from solution ($p < 0.04$), 300 nm ($p < 0.02$) and 12,000 nm ($p < 0.03$), does not differ from 900 nm ($p > 0.12$) and 900 nm + TPP ($p > 0.09$).

added TPP, however, did not show significantly different P_{app} values compared to those without TPP. Therefore TPP was not responsible for the increased permeation enhancement. The largest particles of 12,000 nm are not of practical relevance, as they are too big to cross the BBB, but they are of academic interest to study and understand the size dependency of permeation enhancement.

The measurement of the TEER is an easy and quick method to determine the tight junction integrity (Kotze et al., 1998). In parallel to the permeation experiment the TEER of the cells was measured (Fig. 3). Before the experiment the TEER of all samples was the same and TEER in wells with cells without polymer did not change during or after the experiment. TEER of cells treated with chitosan both in solution and as particles decreased significantly to 10–20% of the initial value during permeation studies. After washing and incubating the cells with DMEM for 24 h TEER increased again. This recovery was more intense in monolayers treated with chitosan LV solution and 12,000 nm particles than with the smaller particles. A decrease in TEER during the permeation experiment can be explained by the modulation and opening of the tight junctions caused by the permeation enhancer chitosan. Lacking recovery of the barrier properties in vivo can be a problem as substances could reach and badly damage the brain. TEER does not completely recover after removal of chitosan at the end of the experiment which is consistent with previous studies (Shah et al., 2007). This is believed to be due to incomplete removal of chitosan from the cell surface caused by bioadhesion and viscosity of the polymer (Smith et al., 2004). However, cell damage should be also taken into account, as the TEER of cells treated with small chitosan particles only recovers to some extent.

Two different tests were performed in order to study toxicity. On one hand the RBC lysis was investigated for blood compatibility and on the other hand LDH release was studied for MDCK cell toxicity. RBC lysis test was performed with different concentrations of chitosan LV solution and particles. Chitosan LV in solution showed in a concentration of 5 mg/ml still only comparatively very low hemolysis as illustrated in Fig. 4. The smaller the particles were the more hemolysis they caused. Addition of TPP has no significant influence on cell lysis.

Results of the LDH test (Fig. 5) were in good correlation with the results of the RBC lysis test. With chitosan LV in solution only relative low LDH release and therefore low cell toxicity was found. All particles displayed much higher cell toxicity than the solution. Bigger particles led to less toxicity than smaller particles. The smaller the particles are the larger is their surface area and therefore the area with that they can interact with cells. The smallest particles can additionally be taken up into cells via endocytosis. Addition of TPP increased the toxicity of 12,000 nm particles but not significantly of 900 nm particles.

4. Conclusion

Within this preliminary study the potential of chitosan to enhance the drug transport across the blood brain barrier has been explored in a cell culture model. The molecular weight dependent permeation enhancement was evaluated and particles of different sizes with the most appropriate chitosan were prepared. The smaller the particles prepared with chitosan LV were, the more increased was the permeation enhancement of low permeable FD4

transport. All particles at a physiological pH of 7.4 showed a higher transport than chitosan solution at pH 6.4. Therefore chitosan particles seem to be suitable for enhancing the transport across the blood brain barrier.

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