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Self-assembling PEG-*p*(CL-co-TMC) copolymers for oral delivery of poorly water-soluble drugs: a case study with risperidone

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Abstract

Diblock PEG-*p*(CL-co-TMC) [methoxypoly(ethylene glycol)-poly(caprolactone/trimethylene carbonate)] copolymers form micelles spontaneously and significantly increase the solubility of poorly water-soluble drugs. The aim of this work was to assess these diblock copolymers as oral drug delivery systems in both in vitro and in vivo experiments using risperidone as a model drug. The permeation of risperidone through Caco-2 cell monolayers showed that the apparent permeation coefficient (Papp) was slightly reduced when risperidone was formulated with the copolymer. Based on the higher apparent drug solubility, the copolymer increased drug flux or the total amount of drug which crossed the Caco-2 monolayers. The Papp of the micelle formulation was higher at 37 °C than at 4 °C. After oral administration to rats, the pharmacokinetic parameters and the pharmacological effect were evaluated. Time courses of receptor occupancy by risperidone after oral administration were similar when risperidone was encapsulated in PEG-*p*(CL-co-TMC) micelles or solubilized in an aqueous tartaric acid vehicle. The areas under the curve (AUC) were not significantly different although the maximal concentration (Cmax) was twofold lower with the copolymer. The polymeric micelles of PEG-*p*(CL-co-TMC) seem to be a good candidate for oral drug delivery of poorly soluble drugs.

Keywords: Self-assembling polymeric micelles; Risperidone; Caco-2 cells; PEG-*p*(CL-co-TMC); Oral delivery

1. Introduction

Many existing and new therapeutic entities exhibit poor water-solubility. Consequently, the delivery of these drugs may become a real challenge. The problem of low solubility has important consequences: it induces poor and variable drug absorption and

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may be a significant impediment to effective pharmaceutical development [1].

Absorption of orally administered drugs, a major determinant for bioavailability, is mainly determined by membrane permeability and drug solubility in the intestinal lumen. Micronization (particle size reduction), complexation, formation of a solid solution, preparation of amorphous solid oral dosage forms, lipid-based systems (microemulsions, emulsions, liposomes), surfactant and polymer micelles, and polymer-based systems (microparticles, microcapsules) are among the many methods proposed to improve the oral bioavailability of poorly water-soluble drugs [2]. In addition, researchers had hoped to find a possibility to use liposomes as universal containers for targeted transport of various biologically active substances in an organism. Studies during the past decade have revealed that serious limitations are linked to this technology: thermodynamic instability, limited storage stability, and poor incorporation efficiency for many drugs. The problem may be solvable using alternative concepts including self-assembling supramolecular complexes. Such complexes should have the following major properties: (i) the complex should form spontaneously; (ii) for effective penetration into tissues, the complex must cross biological barriers, i.e., the size of the complex should not exceed the size of a virus; (iii) before reaching the target cell, the complex should be stable and biologically inert; (iv) after interaction with the target, the complex has to be easily eliminated from the organism. The phenomenon of spontaneous assemblage is a characteristic of most micellar colloidal systems including polymeric micelles.

Polymeric micelles have been proposed as drug carriers due to multiple potential advantages. They can be used for solubilization, stabilization, and delivery of drugs [3–10]. Their hydrophobic core increases the solubility of lipophilic, poorly water-soluble drugs whereas the hydrophilic corona allows a long circulation of the drugs. The corona prevents the interactions between the core and the blood components and allows for steric stabilization. Different methods can be used to incorporate drugs into the core of the micelles such as chemical binding, physical entrapment, or complexation. The incorporation of drugs into diblock polymeric micelles is usually a long, multistep procedure, which often requires the use of organic solvents. These complexities often limit the use of these otherwise beneficial systems [3–10].

As their diameter is usually under 100 nm, polymeric micelles are able to pass the microvasculature. Because of these properties, polymeric micelles have been studied extensively for cancer therapy. Indeed, targeting of anticancer drugs entrapped in polymeric micelles was observed as a consequence of their long circulating time and enhanced permeation and retention effect (EPR) in tumors [11,12]. They have also been shown to reduce toxicity and side effects of drugs [11]. It has also been found that polymeric micelles could protect compounds against enzymes as in the case of ODN complex formed with PEG-poly(L-lysine) diblock polymers which protect ODN's against deoxyribonuclease [13]. Some polymeric micelles can allow a better distribution of the drug to the brain and can inhibit drug efflux pumps such as *P*-gp [14]. However, the use of polymeric micelles or copolymers forming in situ polymeric micelles for oral administration of drug has not been studied.

We have recently developed novel self-assembling diblock methoxypoly(ethylene glycol)-poly(ϵ-caprolactone-co-trimethylene carbonate) (PEG-*p*(CL-co-TMC)) copolymers [15]. These copolymers form micelles spontaneously at dilutions above a concentration of 10 μg/ml and strongly enhance the solubility of poorly water-soluble drugs. We hypothesized that PEG-*p*(CL-co-TMC) may be applicable to oral drug delivery. Consequently, the aim of the present work was to assess whether PEG-*p*(CL-co-TMC) could be used as a potential oral delivery system. Risperidone was selected as a model drug as this drug has a good permeability but low water-solubility (Class II in the biopharmaceutical classification system (BCS)). The potential use of PEG-*p*(CL-co-TMC) micelles for the oral delivery of risperidone was studied in vitro by evaluating the toxicity to Caco-2 cells and by measuring the transport of risperidone loaded micelles across Caco-2 monolayers as this model is the most commonly used to estimate in vitro intestinal drug permeability [16]. Moreover, the risperidone-loaded micelles were tested in vivo in a brain receptor occupancy pharmacodynamic study and pharmacokinetic study [17].

2. Materials and methods

2.1. Copolymers

The PEG-*p*(CL-co-TMC) diblock copolymers were synthesized by the Johnson and Johnson Center for Biomaterials and Advanced Technologies (Somerville, NJ, USA). Caprolactone (CL) was purchased from Union Carbide (Danbury, CT, USA), trimethylene carbonate (TMC) from Boehringer Ingelheim (Petersburg, VA, USA), monomethyletherPEG 750 from Fluka (Milwaukee, WI, USA), and stannous octoate from Aldrich (Milwaukee, WI, USA) [15].

Synthesis follows a ring-opening polymerization process [15]. The copolymer composition and residual monomer content were analysed by proton NMR using a Unity-plus 400 spectrometer from Varian (Palo Alto, CA, USA). The residual monomer content was lower than 1 mol%. The copolymer contained 49±1 mol% of CL and 49±1 mol% of TMC. Gel permeation chromatography (GPC) performed with a Waters Alliance 2690 system (Milford, MA, USA) was used to determine the molecular weight and the polydispersity of the copolymers (MW=5320±505 and PD=1.9±0.1; n=4).

2.2. Preparation of polymeric micelles

Polymeric micelles based on the liquid diblock copolymer of PEG-*p*(CL-co-TMC) were obtained by mixing under gentle stirring, at 37 °C, the diblock copolymer with risperidone before adding purified water. Risperidone was provided by Janssen Pharmaceutica (Beerse, Belgium). Tartaric acid was purchased from Acros Organics (Geel, Belgium). Formulations of risperidone in tartaric acid (0.625%) were obtained by solubilizing the drug with distilled water by salt formation [17,18].

2.3. Caco-2 experiments

2.3.1. Cytotoxicity Assays

Caco-2 cells obtained from ATCC (American type culture collection, USA) were seeded in 96-well plates at a density of 10,000 cells per well. Two hundred microliters of Eagle's minimum essential medium (MEM) supplemented with non-essential amino acids, 2 mM of L-glutamine, 10% of fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 U/ml) was used as growth medium (Invitrogen Life Technologies, Merelbeke, Belgium). Cells were incubated for 48 h at 37 °C under 5% CO_2. The culture medium was then replaced with 180 μl of a solution of the polymeric micelles (1% or 10% w/v polymer) in Krebs buffer. Triton X-100 (1% w/v) was used as positive control while isotonic Krebs buffer was used as the vehicle control. The cells were incubated at 37 °C for 45 min before determining the metabolic activity of each well by a colorimetric assay. After removal of the solutions, 25 μl of a 5 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma, Bornem, Belgium) stock solution in PBS buffer was added to each well. Cells were again incubated for 4 h at 37°C. The media was removed from all wells and 25 μl of a glycine buffer (BioRad, Richmond, CA, US) was added just prior to the addition of 100 μl of the extraction buffer (DMSO; Merck, Darmstadt, Germany) to dissolve the formazan by-product. After 10 min of shaking, the absorbance was measured at 540 nm using a microplate reader (TECAN, Mechelen, Belgium). All plates were analysed within 30 min. From the absorbance, the percent viability of the cells was calculated using the Krebs solution as reference (100% of viability) [19].

2.3.2. Caco-2 cell culture and permeation studies

The Caco-2 cells were used which had a passage number between 40 and 50 for the experiments. They were maintained in DMEM (Dulbelcco's minimal essential medium) supplemented with nonessential amino acids, 2 mM of L-glutamine, 10% of fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 U/ml). All chemical reagents used for culturing the Caco-2 cells were purchased from Invitrogen Life Technologies (Merelbeke, Belgium). Cells were plated at a density of 40,000 cells/cm^2 on Costar Snapwell membrane inserts (polycarbonate filter, diameter of 12 mm and pore size of 0.4 μm). Cells were used for the transport experiments 21 days post-seeding [16,20].

Cells were washed three times for 30 min with Krebs buffer, pH 7.4, with 0.5 ml on the top of the membrane and 2.5 ml below the insert. The different formulations containing risperidone were applied on

the donor side (0.5 ml) in presence or absence of 100 μM verapamil, whereas in the receptor side, 2.5 ml of buffer were applied (Sigma, Bornem, Belgium). Permeation was evaluated in both directions (apical to basolateral and basolateral to apical).

2.3.3. TEER measurement

Transepithelial electrical resistance (TEER) values were measured using a Voltohmmeter EVOM (World Precision Instrument, Hertfordshire, England). TEER of the cell monolayers was controlled 30 min before starting the experiment and during the permeation experiment at 30, 60, 90, 120, and 150 min. Only monolayers with initial values measured 30 min before the experiment higher than 450 Ω cm^2 were used. TEER during the experiment is expressed as the percentage of the initial value.

2.3.4. Paracellular transport

To evaluate the paracellular transport, sodium fluorescein (Fluorescein, Across, Beerse, Belgium) at a concentration of 1 mg/ml was added to the apical compartment at the end of the permeation experiment. After 60 min, the amount of the fluorescein on the basolateral side was analyzed using a fluorescence microplate reader (TECAN, Mechelen, Belgium) [21].

2.3.5. HPLC analysis

The amount of risperidone crossing the Caco-2 monolayer was determined using a high-performance liquid chromatography (HPLC) system consisting of a Waters 2690 Separation module and a Waters 996 photodiode Array detector, equipped with a Hypersil RP18 BDS C18 column. The mobile phase consisted of an aqueous phase of tetrabutylammoniumhydrogensulphate (0.007 M) and an organic phase of acetonitrile (40:60, v/v). The injection volume was 25 μl and elution was performed at a flow rate of 1.5 ml/min. The wavelength of the detector was 273 nm. The detection limit of risperidone was 1 μg/ml [22]. The intraday variability using the HPLC method showed a relative standard deviation of 0.03% for 12.5 μg/ml. The interassay variance was less than 4% for 12.5 μg/ml. The calibration curves were linear (r^2>0.999) over the concentration ranged studied (1.5 to 100 μg/ml).

2.3.6. Analysis of the permeation experiments through Caco-2 monolayer

Results of the permeation experiments through Caco-2 monolayer are expressed as the cumulated amount of the drug permeated through the membrane vs. time.

The apparent permeability coefficient Papp, was calculated using the following equation:

$$Papp = \frac{\Delta Q}{\Delta t} \times \frac{1}{A \times C_0}$$

where $\frac{\Delta Q}{\Delta t}$ is the amount of drug appearing per time unit (t) in the acceptor compartment (basolateral side if the formulations were applied on apical side and apical side if the formulations were applied on basolateral side). The transport rate was calculated by plotting the amount of the drug transported to the acceptor side vs. time and then determining the slope of the plot. C_0 is the initial concentration in the donor compartment (μM). A is the surface area of the Caco-2 monolayer (A=1.13 cm^2) [16]. Results are expressed as a mean±S.D. (n=6).

2.3.7. Statistics

All experiments were conducted at least in triplicate. Significance was tested using one-way ANOVA for the cytotoxicity assay. For all other experiments, either Tukey–Kramer test or Two-tailed t-test was used. Values of p<0.05 were considered statistically significant.

2.4. In vivo receptor occupancy by risperidone

2.4.1. Oral administration and tissue preparation

Male Wistar rats (~200 g, Charles River, Belgium) received 2.5 mg/kg risperidone solubilized at 2.5 mg/ml in 0.625% w/v tartaric acid, or in 10% w/v PEG-*p*(CL-co-TMC) polymeric micelles. Three animals per time point were sacrificed by decapitation at 10 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h, 24 h after administration of the two risperidone formulations. Heparinized blood samples were collected from every animal. Brains were immediately removed from the skull and rapidly frozen in dry ice-cooled 2-methylbutane (−40 °C). 20-μm-thick frontal sections were cut using a Leica CM 3050 cryostat–microtome and

thaw-mounted on adhesive microscope slides (Star Frost, Knittel Gläser, Germany). The sections were kept at −20 °C until use [18].

2.4.2. Ex vivo radioligand binding in brain sections

Occupancy of D_2 receptors by risperidone was measured in the striatum of each rat using [^{125}I] iodosulpride (Amersham Pharmacia, UK) as the radioligand. Occupancy of 5-HT_{2A} receptors by risperidone was measured in the frontal cortex of each rat using [^{3}H] ketanserine (Perkin–Elmer, Belgium) as the radioligand. Ex vivo receptor occupancy was measured according to the protocol described by Schotte et al. [18]. The sections were not washed prior to incubation, to avoid dissociation of the drug-receptor complex. Brain sections from drug-treated and vehicle-treated animals were incubated in parallel for 10 min with 0.2 nM iodosulpride for D_2 receptors and 1 nM ketanserine for 5-HT_{2A}. After incubation, the excess radioligand was washed off using ice-cold buffer, followed by a quick rinse in cold distilled water. The sections were then dried under a stream of cold air, placed in a light-tight cassette and covered with films (Kodak Ektascan GRL films for [^{125}I] and Amersham [^{3}H] Hyperfilms for [^{3}H]). After 1 week of exposure for [^{125}I] and 4 weeks of exposure for [^{3}H], the films were developed in Kodak X-Omat processor.

2.4.3. Quantitative autoradiography analysis

Autoradiograms were quantified using an MCID (micro computer imaging device) image analyzer (Imaging Research, St-Catharines, Ontario, Canada). Optical densities were transformed into levels of bound radioactivity after calibration of the image analyzer with grey-values generated by coexposure of commercially available radioactive standards ([^{125}I] and [^{3}H] Micro-scales, Amersham) with the tissue sections. Specific binding was calculated as the difference between total binding and non-specific binding. Ex vivo receptor labeling by the radioligand in brain sections of drug-treated animals is expressed as the percentage of receptor labeling in the corresponding brain sections of vehicle-treated animals. Since only unoccupied receptors remain available for the radioligand, ex vivo receptor labeling is inversely proportional to the receptor occupancy by the in vivo administered drug. Percentages of receptor occupancy by the drug administered to the animal corresponded to 100% minus the percentage of receptor labeling in the treated animal [18].

At each time point, an unpaired *t*-test was performed for evaluating differences in receptor occupancy for both formulations of risperidone.

2.4.4. LC-MS/MS method for risperidone and 9-hydroxy-risperidone in rat plasma

The blood collected after decapitation was centrifuged before determining the concentration of risperidone and its metabolites in the plasma by LC-MS/MS [23]. Prior to LC-MS/MS analysis, risperidone and its metabolites were isolated from the plasma by a solid phase extraction. In brief, to plasma samples (0.1 ml aliquots), 2 ml of 0.1 M phosphate buffer pH 6.0 was added followed by the addition of an internal standard (internal risperidone solution) and 100 μl of methanol. Before applying the samples on a Bond Elut Certify column (10 cm^3, SPE Varian, Sint-Katelijne Waver, Belgium), 1 ml of 0.1 M phosphate buffer was added to each sample. The columns were washed with water, 1 M acetic acid, and methanol prior to elution of the active molecules with 3 ml methanol-NH_4OH (25%; 98/2 v/v). After evaporation of the samples to dryness, the residues were reconstituted in 300 μl 0.01 M ammonium formate pH 4.0/Acetonitrile (50/50 v/v). After vortexing, 20 μl aliquots of the supernatant were injected onto a reversed-phase LC-column (Hypersil BDS C18 3 μm, 100×4.6 mm; Alltech, Lokeren, Belgium) [23].

LC-MS/MS analysis was carried out on an API-3000 MS/MS (Applied Biosystems, Belgium), which was coupled to an HPLC-system (Agilent Technologies, Diegem, Belgium). The MS/MS, operated in the positive ion mode using the TurboIonSpray™-interface (electrospray ionization) and was optimized for the quantification of risperidone, 9-hydroxyrisperidone, and the internal standards. The limit of quantification defined as the lowest spiked standard of the calibration curve, which was prepared in the same matrix as the study samples, was 0.5 ng/ml for the two compounds. Linearity of the calibration curves ranged from 0.5 to 1250 ng/ml for the two compounds. The accuracy (inter- and intrabatch) accuracy was between 85% and 115% of the nominal value over the entire range. The precision was within 15% over the entire range.

3. Results and discussion

3.1. Cytotoxicity of PEG-p(CL-co-TMC)

The successful pharmaceutical exploitation of self-assembling polymeric micelles will depend on several factors including their safety, loading efficiency and their ability to delivery poorly water-soluble drugs after administration. In order to gauge toxicity, a number of in vitro assessments were completed. The cytotoxicity of the copolymers of interest (i.e., the PEG-*p*(CL-co-TMC)) was measured in Caco-2 cells using (1) the colorimetric MTT test to assess viability, (2) the measurement of TEER values to assess tight junctional integrity, and (3) the assessment of sodium fluorescein flux to investigate the possible effect of the copolymers on paracellular transport characteristics and integrity.

The cytotoxicity of the diblock copolymeric micelles without an incorporated drug was evaluated using a microtiter well protocol in which Caco-2 cells were exposed to various concentrations of the copolymers over a 4-h time course. Concentrations of 1% and 10% w/v PEG-*p*(CL-co-TMC) in buffer did not affect the viability of the cells. Specifically, a viability of at least 85% was measured for the polymeric formulations whereas the cells incubated in presence of 1% Triton X-100 exhibited a viability of less than 15% (Fig. 1; $p<0.05$ with Triton X-100 vs. Krebs buffer control). No significant change was observed when the concentration of the copolymer was increased from 1% to 10% ($p>0.05$). Additionally, the PEG-*p*(CL-co-TMC) polymeric solution at concentrations up to and including 10% w/v were not hemolytic in vitro (data not shown).

To assess whether the PEG-*p*(CL-co-TMC) copolymers could affect paracellular transport by opening the tight junctions between cells, the influence of the polymeric micelles on TEER and the fluorescein flux through Caco-2 monolayers was measured (Fig. 2) [24]. In these studies, the transport of the model drug, risperidone, was measured. In both the polymeric micelles and a nonmicellar formulation (i.e., a solution of risperidone in tartaric acid), the TEER measured at the end of the permeation experiment showed a reduction of only 20% of the initial value. The fluorescein flux was approximately 0.6% and was essentially unchanged in the presence or absence of the copolymer [21]. These results indicate that the polymeric micelles did not affect the tight junctions and the permeability of the monolayer to ionic conductance. These data suggest that the polymeric

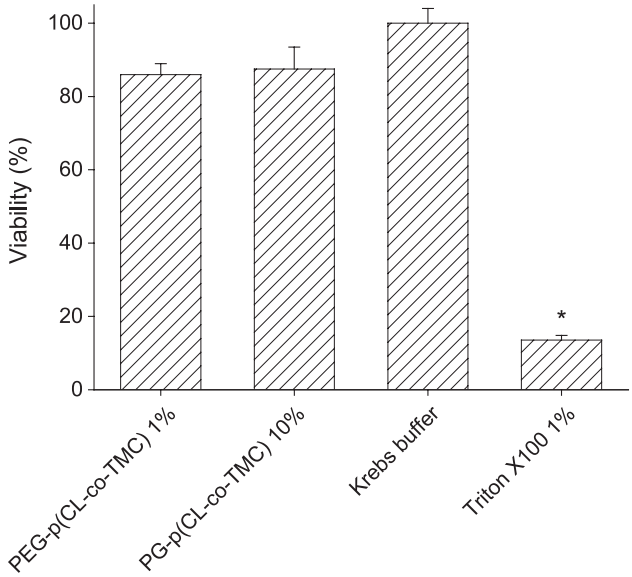

Fig. 1. Cytotoxicity of 1% and 10% w/v PEG-*p*(CL-co-TMC) polymeric solutions to Caco-2 cells. Krebs buffer was used as the vehicle control, while 1% Triton X-100 was the positive control of cell lysis (mean±S.D., $n=6$). (*$p<0.05$ vs. Krebs buffer).

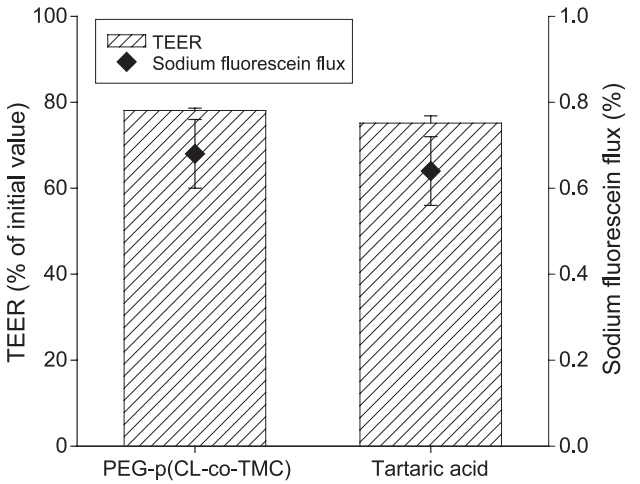

Fig. 2. Effect of risperidone (1 mg/ml) solubilized in 10% w/v polymeric solution or in 0.625% w/v tartaric acid on the TEER values (mean±S.D., n=6) and on sodium fluorescein flux (1 mg/ml) across the Caco-2 monolayer at the end of the experiment (mean±S.D., n=3).

micelles up to concentration of at least 10% are not acutely toxic to Caco-2 cells and can be used to investigate drug permeation in vitro.

3.2. Risperidone transport across Caco-2 monolayers

To assess whether drug transport through cell monolayers was affected by the applied copolymer, the permeability and flux of risperidone was determined using two formulations including one based on the polymeric micelles and one in which risperidone was simply dissolved in an acidic vehicle (tartartic acid). The time course of the absorptive transport of risperidone across the Caco-2 monolayer is shown in Fig. 3. The transport occurred in a linear fashion as a function of time. At equal concentrations, the amount of drug permeating the Caco-2 monolayer was higher when the drug was dissolved in tartaric acid than when it was encapsulated in the polymeric micelles (p<0.05). At saturation, the amount of risperidone permeating was higher with the polymeric micelles because the apparent solubility of the drug was enhanced in accordance with Fick's First Law (Fig. 3). Apparent permeability (Papp) values were in the

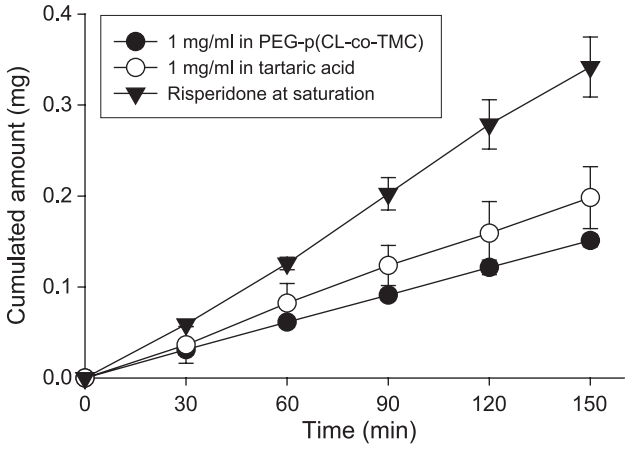

Fig. 3. Permeation of various risperidone formulations through Caco-2 cells. Results are expressed as cumulated amount vs. time (mean±S.D., n=6). (●)=1 mg/ml risperidone in 10% w/v PEG-p(CL-co-TMC) polymeric solution, (○)=1 mg/ml risperidone in 0.625% tartaric acid, (▼)=risperidone saturated in 10% w/v PEG-p(CL-co-TMC) polymeric micelles.

range of 10 to 16×10^{-6} cm/sec for risperidone in the polymeric micelle formulation and 22 to 25×10^{-6} cm/sec using the tartaric acid solution (Figs. 4 and 5). A significant difference ($p<0.01$) was found between the Papp of polymeric micelles and the Papp of tartaric acid solution.

To examine whether carrier-mediated transport is involved in the transepithelial transport of risperidone across Caco-2 monolayers, the bidirectionnal transport of the model drug solubilized in tartaric acid was determined as a function of time. As shown in Fig. 4, the transport of risperidone (dissolved in tartaric acid) was observed to be similar in both directions ($p>0.05$). Incubation of the cells with 100 μM verapamil, an inhibitor for *P*-gp did not affect the Papp ($p>0.05$). These results indicate that the drug permeates by simple passive diffusion and that no *P*-gp-mediated transport mechanism is involved in the transport of risperidone, as such, through Caco-2 monolayers. This last result is in accordance with the in vivo study of Yasui-Furukori et al. [25], which suggest that the *MDR-1* (*MDR-1* is a gene which codes for *P*-gp) variants are not associated with steady-state plasma concentration of risperidone or 9-hydroxyrisperidone.

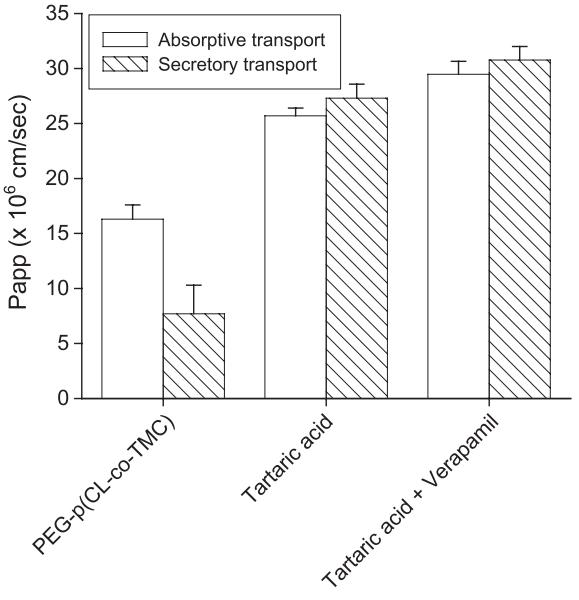

Fig. 4. Bidirectional transport of 0.4 mg/ml risperidone across Caco-2 monolayers. Risperidone was dissolved in 0.625% tartaric acid solution or in 10% w/v polymeric solution. Papp was measured in absence or presence of 100 μM verapamil. Open bars represent the absorptive transport from apical to basolateral side, while the hatched bars represent the secretory transport from the basolateral to apical side (mean±S.D., $n=6$).

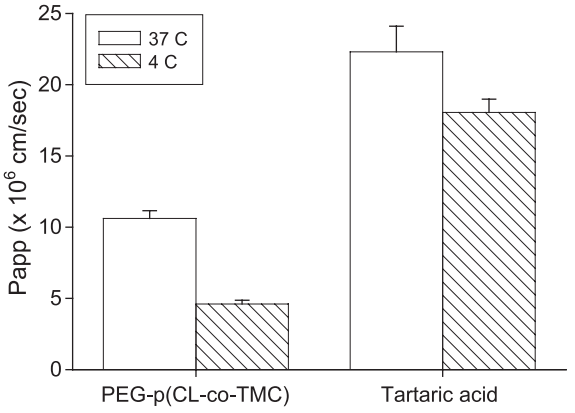

Fig. 5. Influence of temperature (Hatched bars: 4 °C and open bars: 37 °C) on permeation of various risperidone formulations through Caco-2 monolayers: 1 mg/ml risperidone was dissolved in 0.625% w/v tartaric acid or in 10% w/v PEG-*p*(CL-co-TMC) polymeric solution (mean±S.D., $n=6$).

When the drug was entrapped in the polymeric micelles, the Papp in the basolateral–apical direction was three times lower ($p<0.01$) than in the apical–basolateral direction. As the drug is entrapped in the micelles, it could become unavailable for passive diffusion through the monolayers. To further elucidate the transport mechanism of risperidone in presence of the polymeric micelles through the Caco-2 monolayer, permeation experiments were performed at 4 and 37 °C. As shown in Fig. 5, the Papp at 4 and 37 °C were similar when risperidone was solubilized in tartaric acid ($p>0.01$), whereas there was a significant difference ($p<0.01$) when the drug was formulated in the polymeric micelles. Indeed, transport of risperidone formulated in polymeric micelles was more effective at 37 than at 4 °C indicating that the process was temperature-dependent. The uptake of the micelles based on PCL-PEO was severely inhibited by a decrease of temperature from 37 °C to 4 °C [26]. These two pieces of evidence, i.e., transport directionality and temperature-dependent transport, are consistent with active processing including those associated with carriers as well as receptor-mediated endocytosis [27].

3.3. In vivo PK/PD studies on oral delivery of risperidone with PEG-p(CL-co-TMC)

To evaluate the novel formulation based on polymeric micelles as oral drug delivery system in vivo, a pharmacokinetic–pharmacodynamic study was performed. The ability of PEG-*p*(CL-co-TMC) polymeric solutions to deliver risperidone to the brain after oral treatment was evaluated by measuring the central occupancy of dopamine D_2 and serotonin 5-HT_{2A} receptors, the two main therapeutic targets for the antipsychotic drug [17]. In this study, risperidone solution of PEG-*p*(CL-co-TMC) micelles was compared with an aqueous solution of risperidone containing two equivalents of tartaric acid, a standard vehicle for pharmacological studies of risperidone [17]. For both formulations, the maximum dopamine D_2 and serotonin 5-HT_{2A} receptor occupancy was reached 30 min after oral administration (Fig. 6). The level of receptor occupancy remained constant for about 2 h after administration and started to decline at 4 h. Overall, the D_2 and 5-HT_{2A} receptor occupancy profiles of both formulations were very similar. Differences between both formulations reached statistical significance at only one time point and for one receptor investigated. Specifically, at 10 min after administration, the level of 5-HT_{2A} receptor occupied by risperidone was lower when formulated in copolymer (27.7±12%) compared to 62±4% occupancy for risperidone in tartaric acid ($p<0.05$; Fig. 6A and B). At 2 h, similar brain receptor occupancy were observed for a 1% and 10% PEG-*p*(CL-co-TMC) concentration (data not shown).

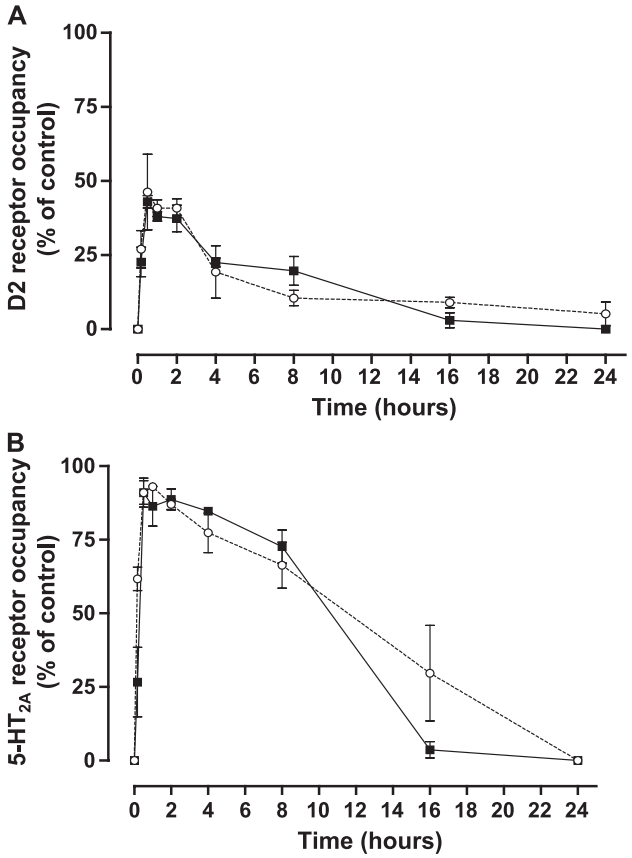

Fig. 6. Kinetics of (A) D_2 and (B) 5-HT_{2A} receptor occupancy in the brain by risperidone after oral administration of 2.5 mg/kg risperidone in 0.625% tartaric acid (○) or in a 10% micellar solution of PEG-*p*(CL-co-TMC) (■) (mean±S.D., $n=3$).

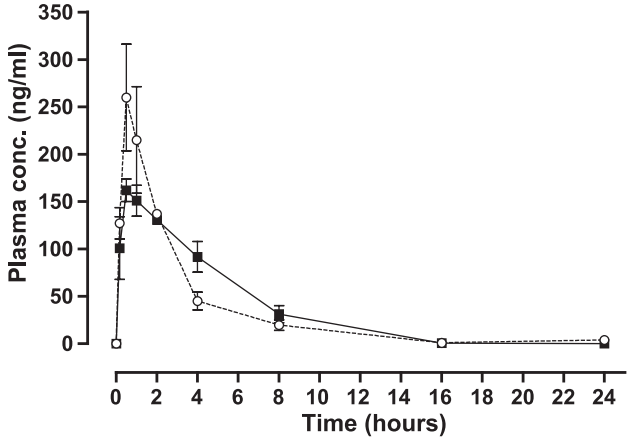

Fig. 7. Plasma profiles of the active moieties (risperidone and 9-OH-risperidone) after the oral administration of 2.5 mg/kg risperidone in 0.625% tartaric acid (○) or a 10% micellar solution of PEG-*p*(CL-co-TMC) (■) (mean±S.D., *n*=3).

The time course profiles of the plasma concentration of risperidone and of 9-OH-risperidone, an active metabolite, are shown in Fig. 7. The peak plasma concentrations were reached at 30 min and immediately started to decline thereafter. Although there were no statistical significant differences between the two formulations due to the limited number of animals used at each time point (n=3), the Cmax of risperidone tended to be lower when formulated in polymeric micelles (162±12 vs. 256±56 ng/ml, mean±S.E.M.; p=0.16; Table 1). The similar areas under the curve (AUC) indicate that the bioavailability was equivalent for both formulations. The fact that, at 4 and 8 h, the three parameters (D_2 occupancy, 5-HT_{2A} occupancy, plasma concentration) tended to be higher when risperidone was administered in polymeric micelles could indicate that the absorption of the drug was sustained. Additional data will be required to validate this observation.

4. Conclusion

The oral delivery of poorly water-soluble drugs is a growing issue for the pharmaceutical industry as many drug candidates resulting from high throughput screening possess these physicochemical limitations. Polymeric micelles are promising drug carriers for these poorly soluble drugs but their potential use as oral drug delivery systems has not been widely demonstrated in vivo. We hypothesized that PEG-*p*(CL-co-TMC) copolymers, which form micelles spontaneously upon dilution and increase drug solubility, could be used as oral drug delivery systems. To assess this hypothesis, we first assessed a number

Table 1
Pharmacokinetic parameters of the active moieties (risperidone and 9-OH risperidone) following oral administration of risperidone solubilized in tartaric acid or in PEG-*p*(CL-co-TMC) micelles

	Tartaric acid			Polymeric micelles		
	Risperidone	9-OH risperidone	Total (active moiety)	Risperidone	9-OH risperidone	Total (active moiety)
AUC (ng min/ml)	ND[a]	ND[a]	46911.7	ND[a]	ND[a]	52218.4
Tmax (min)	30	60	30	30	60	30
Cmax (ng/ml)	119	143	256±56	67	98	162±12
T1/2 (min)	95	160	ND[a]	158	300	ND[a]

[a] Not determined.

of in vitro parameters using Caco-2 monolayers, a conventional drug permeability model. Specifically, the effect of the PEG-*p*(CL-co-TMC) copolymer on the drug permeation of a BCS class II model drug, risperidone, was evaluated. As compared to risperidone solubilized in tartaric acid, the Papp was slightly decreased in the presence of the copolymer. However, the total amount of drug, which could be transported, was increased because the copolymer increases drug solubility.

The mechanism by which risperidone, solubilized in the PEG-*p*(CL-co-TMC), is transported remains to be established. The preliminary data indicate that transport is temperature dependent, suggesting that an active transport such as endocytosis is involved. This endocytosis pathway has been suggested for other polymeric micelles [28,29]. In addition, preliminary confocal microscopy studies with fluorescent probes show punctuated fluorescence in the Caco-2 cytoplasm (data not shown). It has been demonstrated that several surfactants and amphiphilic polymers can inhibit the *P*-gp efflux. However, as risperidone is not a substrate of *P*-gp, we could not determine if PEG-*p*(CL-co-TMC) interacts with this efflux pump. The transport of risperidone from the basolateral to apical side was significantly reduced by the copolymer. This could be explained by a lower endocytosis at the basolateral site.

As a proof of concept that the PEG-*p*(CL-co-TMC) could be used as oral delivery system, an in vivo rat study with the model drug, risperidone, was performed. Brain receptor occupancy and plasma level were compared after intragastric delivery. D_2 and 5-HT_{2A} receptor occupancy were similar when risperidone was formulated either in a solution or with polymeric micelles. The same AUC was measured, indicating a similar bioavailability. However, a reduced Cmax and a higher Tmax suggest that PEG-*p*(CL-co-TMC) micelles could provide a sustained release delivery.

Except for several surfactants of the Pluronics® class, most of the existing amphiphilic polymers forming micelles have been tested after intravenous administration. The fact that PEG-*p*(CL-co-TMC) could be delivered in a capsule to form, upon dilution in the gastrointestinal tract, the drug containing micelles with a low CMC is of particular interest [15]. These novel copolymers could open new opportunities for the oral delivery of poorly soluble drugs.

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References

[1] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (2001) 3–26.
[2] R.G. Strickley, Solubilizing excipients in oral and injectable formulations, Pharm. Res. 21 (2004) 201–230.
[3] P. Alexandridis, B. Lindman, Amphiphilic Block Copolymers: Self-Assembly and Applications, Elsevier, New-York, 2000.
[4] G.S. Kwon, K. Kataoka, Block copolymer micelles as long circulating drug vesicles, Adv. Drug Deliv. Rev. 16 (1995) 295–309.
[5] C. Allen, D. Maysinger, A. Eisenberg, Nano-engineering block copolymer aggregates for drug delivery, Colloids Surf., B Biointerfaces 16 (1999) 3–27.
[6] M.C. Jones, J.C. Leroux, Polymeric micelles—a new generation of colloidal drug carriers, Eur. J. Pharm. Biopharm. 48 (1999) 101–111.
[7] G.S. Kwon, Block copolymer micelles as drug delivery systems, Adv. Drug Deliv. Rev. 54 (2002) 169–190.
[8] A.V. Kabanov, P. Lemeiux, S. Vinogradov, V. Alakhov, Pluronic block copolymers: novel functional molecules for gene therapy, Adv. Drug Deliv. Rev. 54 (2002) 223–233.
[9] M.L. Adams, A. Lavasanifar, G.S. Kwon, Amphiphilic block copolymers for drug delivery, J. Pharm. Sci. 92 (2003) 1343–1355.
[10] N. Rapoport, W.G. Pitt, H. Sun, J.L. Nelson, Drug delivery in polymeric micelles: from in vitro to in vivo, J. Control. Release 91 (2003) 85–95.
[11] M. Yokoyama, T. Okano, Y. Sakura, S. Fukushima, K. Okamoto, K. Kataoka, Selective delivery of adriamycin to a solid tumor using a polymeric micelle carrier system, J. Drug Target. 7 (1999) 171–186.
[12] H.S. Yoo, T.G. Park, Biodegradable polymeric micelles composed of doxorubicin conjugate PLGA-PEG block copolymer, J. Control. Release 70 (2001) 63–70.
[13] A. Harada, H. Togawa, K. Kataoka, Physicochemical properties and nuclease resistance of antisense-oligodeoxynucleotides entrapped in the core of polyion complex micelles composed of poly(ethylene glycol)-poly(L-lysine) block copolymers, Eur. J. Pharm. Sci. 13 (2001) 35–42.

[14] A. Kabanov, E. Batrakova, N. Melik-Nubarov, N. Fedoseev, T. Dorodnich, V. Alakhov, V. Chekhonin, I. Nazarova, V. Kabanov, A new class of drug carriers: micelles of poly (oxyethylene)-poly (oxypropylene) block copolymers as microcontainers for drug targeting from blood in brain, J. Control. Release 22 (1992) 141–158.

[15] L. Ould-Ouali, A. Ariën, J. Rosenblatt, A. Nathan, P. Twaddle, T. Matalenas, M. Borgia, S. Arnold, D. Leroy, M. Dinguizli, L. Rouxhet, M. Brewster, V. Préat, Biodegradable self-assembling PEG-copolymer as vehicle for poorly water-soluble drugs, Pharm. Res. 21 (2004) 1581–1590.

[16] P. Artursson, K. Palm, K. Luthman, Caco-2 monolayers in experimental and theoretical predictions of drug transport, Adv. Drug Deliv. Rev. 46 (2001) 486–491.

[17] A.A.H.P. Megens, F.H.L. Awouters, T.F. Meert, C. Dugovic, C.J.E. Niemegeers, J.E. Leysen, Survey on the pharmacodynamics of the new antipsychotic risperidone, Psychopharmacology 114 (1994) 9–23.

[18] A. Schotte, P.F.M. Janssen, W. Gommeren, W.H.M.L. Luyten, P. Van Gompel, A.S. Lesage, K. De Loore, J.E. Leysen, Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding, Psychopharmacology 124 (1996) 57–73.

[19] T.J. Mossman, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.

[20] I.J. Hidalgo, T.J. Raub, R.T. Borchardt, Characterization of the human colon carcinoma cell line (CaCo-2) as a model system for intestinal epithelial permeability, Gastroenterology 96 (1989) 736–749.

[21] P. Augustijns, R. Mols, HPLC with programmed wavelength fluorescence detection for simultaneous determination of marker compounds of integrity and *P*-gp functionality in the Caco-2 intestinal absorption model, J. Pharm. Biomed. Anal. 34 (2004) 971–978.

[22] L.E.C. Van Beijsterveldt, R.J.F. Geerts, J.E. Leysen, A.A.H.P. Megens, H.M.J. Van den Eynde, W.E.G. Meuldermans, J.J.P. Heykants, Regional brain distribution of risperidone and its active metabolite 9-hydroxy-risperidone in the rat, Psychopharmacology 114 (1994) 53–62.

[23] B.M. Remmerie, L.L. Sips, R. de Vries, J. de Jong, A. Schothuis, E.W. Hooijschuur, N. Van de Merbel, Validated method for the determination of risperidone and 9-hydroxy-risperidone in human plasma by liquid chromatography-tandem mass spectrometry, J. Chromatogr., B, Biomed. Sci. Appl. 763 (2003) 461–472.

[24] S. Deferme, R. Mols, W. Van Driessche, P. Augustijns, Apricot extract inhibits the *P*-gp-mediated efflux of talinolol, J. Pharm. Sci. 12 (2002) 2539–2548.

[25] N. Yasui-Furukori, K. Mihara, T. Takahata, A. Suzuki, T. Nakagami, R. De Vries, Effects of various factors on steady-state plasma concentrations of risperidone and 9-hydroxy-risperidone: lack of impact of MDR-1 genotypes, Br. J. Clin. Pharmacol. 57:5 (2004) 569–575.

[26] C. Allen, Y. Yu, A. Eisenberg, D. Maysinger, Cellular internalization of PCL_{20}-*b*-PEO_{44} block copolymer micelles, Biochim. Biophys. Acta 1421 (1999) 32–38.

[27] J.N. Cogburn, M.G. Donovan, C.S. Schasteen, A model of human small intestinal absorptive cells: 1. Transport barrier, Pharm. Res. 8 (1991) 210–216.

[28] A.V. Kabanov, E.V. Batrakova, V.Y. Alakhov, Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery, J. Control. Release 82 (2002) 189–212.

[29] J. Zastre, J. Jackson, M. Bajwa, R. Liggins, F. Iqbal, H. Burt, Enhanced cellular accumulation of a *P*-glycoprotein substrate, rhodamine-123, by Caco-2 cells using low molecular weight methoxypolyethylene glycol-*block*–polycaprolactone diblock copolymers, Eur. J. Pharm. Biopharm. 54 (2002) 299–309.