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Pluronic block copolymers and Pluronic poly(acrylic acid) microgels in oral delivery of megestrol acetate

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Abstract

Several Pluronic-based formulations were studied in-vitro and in a rat model with respect to the release and bioavailability of megestrol acetate (MA) after oral administration. It was demonstrated that an aqueous, micellar formulation comprising a mixture of a hydrophobic (L61) and a hydrophilic (F127) Pluronic copolymer, significantly enhanced the bioavailability of MA administered orally at relatively low doses (1–7 mg kg^{-1}). Pluronic-based microgels (spherical gel particles of sub-millimetre size) were introduced as MA vehicles. The microgels comprised a cross-linked network of poly(acrylic acid) onto which the Pluronic chains were covalently attached. Microgels of Pluronic L92 and poly(acrylic acid) fabricated into tablet dosage forms exhibited dramatically lowered MA initial burst release. The MA release was pH-dependent owing to the pH sensitivity of the microgel swelling, with the drug retained by the microgel at pH 1.8 and released slowly at pH 6.8. In the rat model, a significant increase in MA bioavailability was observed when the microgel-formulated MA was administered orally at a high dose of 10 mg kg^{-1}, owing to the enhanced retention of the microgel. The study of the microgel passage through the gastrointestinal tract demonstrated the microgel retention characteristic of a very high molecular weight polymer and the absence of any systemic absorption of the polymer.

Introduction

Oral drug delivery is one of the most convenient routes of drug administration. However, several major factors are known to prevent drugs from entering the bloodstream after oral administration. These include poor aqueous solubility that limits the potential of the drug interaction with the intestinal tissues, drug efflux mediated by ATP binding cassette transporters such as P-glycoprotein (P-gp) and other protein pumps, and drug degradation by metabolic enzymes in the intestinal and hepatic compartments (Sood & Panchagnula 2001; Mahato et al 2003; Chan et al 2004). Numerous technologies aimed at addressing selected problems in enhancing the oral drug availability have been developed. The drug solubility can be enhanced by using nano-sized crystals and particles (Kreuter 1996; Allemann et al 1998; Muller et al 2001; Merisko-Liversidge et al 2003; Sethia & Squillante 2003) and surfactant- and oil-based microemulsions (Gershanik & Benita 2000). Drug metabolism can be reduced by pulsatile and delayed release systems (Bussemer et al 2001; Verma et al 2002; Vasir et al 2003) and modulation of drug efflux by using modulating agents or absorption enhancers (Kimura et al 2002; Kruijtzer et al 2002, 2003). Although these approaches are effective in improving many oral drug forms, a considerable number of valuable pharmaceuticals still need a broader solution that would address the complex combination of factors involved in drug transport from the gastrointestinal tract to the bloodstream. For example, combining a sustained release system that allows control of the kinetics of drug absorption via optimization of the drug residence time at the absorption site, with an absorption enhancer that increases the drug absorption rate, would provide an efficient formulation capable of enhancing the oral bioavailability of compounds, the intestinal absorption of which is limited by a certain number of receptors expressed on the surface intestinal barrier. One example of such compounds is megestrol acetate (MA), which is known to

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have a low and poorly controllable bioavailability owing to P-gp mediated efflux (Yang et al 1989; Fleming et al 1992; Lum et al 1993; Wang et al 1994; Panasci et al 1996; Tansan et al 1997; Eltabbakh et al 1999; Markman et al 2000). This drug is a synthetic analogue of progesterone and is widely used for the palliative treatment of advanced breast and endometrial carcinomas (Martindale & Reynolds 1993). Other indications include the treatment of ailments associated with HIV infection (Von Roenn et al 1988; Tchekmedyian et al 1991) and weight control in the elderly (Raney et al 2000). While the dosing of MA can be up to 800 mg per day, the oral bioavailability of this drug is low and limited by its negligible aqueous solubility (Farinha et al 2000) and P-gp mediated efflux (Yang et al 1989; Fleming et al 1992; Lum et al 1993; Wang et al 1994; Panasci et al 1996; Tansan et al 1997; Eltabbakh et al 1999; Markman et al 2000). The latter factor is likely to be responsible for a high inter-patient variability in MA absorption (Camaggi et al 1995).

We have previously reported that poly(ethylene oxide) and poly(propylene oxide) block copolymers (trade name Pluronic) are potent modulators of the P-gp (Alakhov et al 1996; Venne et al 1996) and other ATP binding cassette transporters, as well as metabolic detoxification enzymes such as glutation-*S*-transferase (Batrakova et al 2003a). Furthermore, we have previously shown that Pluronic copolymers can significantly enhance the transcellular transport of various drugs across intestinal epithelium by inducing a highly selective and transient ATP depletion in this tissue (Batrakova et al 2003b). Structure–activity relationship studies demonstrated that Pluronic polymers with the molecular mass of the poly (propylene oxide) block exceeding 50% of the total molecular mass of the macromolecule are the most potent in modulating the transporters activity (Kabanov et al 2003). The Pluronic copolymers L92 and L61 (average compositions $EO_3PO_{30}EO_3$ and $EO_8PO_{52}EO_8$, respectively) were shown to be the most efficient in modulating P-gp mediated drug efflux. The formulation containing mixtures of aqueous solutions of Pluronic F127 (average formula $EO_{99}PO_{67}EO_{99}$) and L61 has been previously tested in cell-based models and animal models with several drugs such as doxorubicin (Alakhov et al 1999), plasmid DNA (Lemieux et al 2000) and others, including campthotecins, taxanes and podophilotoxins. The composition comprising a mixture of Pluronic F127 and L61 was found to be optimal in terms of its solubility, toxicity pattern and P-gp modulation activity, and is presently being evaluated in several oncology clinical trials (Danson et al 2004). We predicted that utilization of Pluronic copolymers would enhance MA bioavailability through the above-mentioned mechanisms.

In the present study, we explored the useful properties of Pluronic copolymers, along with the capability of their aqueous micellar solutions to solubilize hydrophobic agents (Hurter et al 1995), for oral administration of MA. In order to further enhance the potential of the Pluronic-based formulations for oral delivery of MA, we investigated the behaviour of the Pluronic copolymers covalently cross-linked to a pH-dependent bioadhesive component, poly(acrylic acid) (PAA). Pluronic-PAA microgel particles have been recently introduced as a new type of drug delivery vehicle (Bromberg et al 2002, 2003). The characteristic size of these particles is typically 1–10 μm in the dry state and 50–200 μm in the swollen state. Structurally, the microgels consist of a lightly cross-linked PAA network with dangling Pluronic chains, which can assemble into aggregates within the gel microparticle. The aggregates act analogously to the micelles in the parent Pluronic nanodispersions and are capable of solubilizing hydrophobic agents such as steroid hormones, taxol and camptothecins (Bromberg et al 2003). In addition to the solubilizing capacity, the microgels are mucoadhesive, a property potentially useful in oral drug delivery. Therefore, in the present work we set out to evaluate oral bioavailability of both Pluronic solutions and microgel-based formulations of MA.

Materials and Methods

Materials

Pluronic F127, L92 and L61 were obtained from BASF Corp. (Mount Olive, NJ, USA) and used without further treatment. Acrylic acid (99+%), lauroyl peroxide (97%), 4′,4-azobis-(4-cyanovaleric acid) (98+%), dodecane (99+%), hexane, sodium dodecyl sulfate (SDS), MA and ethylene glycol dimethacrylate (98%) were purchased from Sigma-Aldrich Canada Ltd (Oakville, ON, Canada) and used as received, except for the acrylic acid, which was purified from the inhibitor (4-methoxyphenol) by passing through a molecular sieve column. Poly(vinylpyrrolidinone-co-1-hexadecene) (Ganex V-216) (dispersion stabilizer) was a gift from ISP Technologies, Inc. (Wayne, NJ, USA) and used as received. Methylcellulose (Methocel A4M PREM) was kindly donated by Dow Chemical Company (Midland, MI, USA). [2-^{14}C] Acrylic acid was synthesized from [2-^{14}C]malonic acid (Amersham Biosciences Corp, Piscataway, NJ, USA) as described in detail by Riley et al (2001a). A total of 0.2 g (~5.9 MBq) of the ^{14}C-labelled acrylic acid was synthesized, mixed with 25 mL of inhibitor-free acrylic acid and the mixture stored at −8°C. All other chemicals, solvents and gases were obtained from commercial sources and were of the highest purity available.

Microgel synthesis

Microgels composed of PAA grafted with Pluronic L92 and cross-linked by ethylene glycol dimethacrylate were synthesized by dispersion/emulsion polymerization as described previously (Bromberg et al 2002). Briefly, acrylic acid (40 g) was partially neutralized by addition of 5 M NaOH solution (0.5 mL) under constant agitation and nitrogen flow. Pluronic (24 g) was then allowed to dissolve in the acrylic acid under constant agitation and nitrogen flow. A multi-necked flask equipped with a mechanical stirrer, thermometer, heating bath, and gas inlet/outlet was filled with 250 mL of 1% Ganex solution in dodecane

and was deoxygenated for 1 h under constant agitation and nitrogen flow. An initiator system comprising a solution of lauroyl peroxide (100 mg) and 4′,4-azobis-(4-cyanovaleric acid) (100 mg) in 5 mL of acrylic acid was added to the solution of Pluronic in partially neutralized acrylic acid. Ethylene glycol dimethacrylate (1.1 mL) was added to the resulting solution and the latter was deoxygenated for 1 h under constant agitation and nitrogen flow. The reactor was heated to 70°C at a rate of 1°C min^{-1}. To complete the polymerization reaction, the reactor was maintained at 70°C for 10 h and the contents were filtered using Whatman filter paper 5 (Whatman International, Ltd, Maidstone, KY, USA). The resulting polymeric particles were repeatedly washed with hexane and dried in vacuum. Radioactively labelled microgels were synthesized using the [2-^{14}C]acrylic acid via the same procedure scaled down 10-fold.

Preparation of MA formulations

MA formulation with microgel

Microgel particles (500 mg of dry powder) were suspended in 20 mL of anhydrous ethanol and were allowed to equilibrate for 1 h. MA (100 mg) was added to the suspension, and the mixture was gently stirred at room temperature overnight, forming a homogenous, opaque liquid. The solvent was removed in a stream of dry nitrogen and the process of drying was completed using a vacuum (0.1 Torr) for 48 h. The powders resulting from passing the formulation through a Mesh 40 sieve ('Mesh' Series; Fisher Scientific, Nepean, ON, Canada) were characterized by a particle size less than 425 μm.

MA formulation with Carbopol

The same procedure as described above was used to prepare MA formulation with Carbopol 974P NF (Noveon, Inc., Cleveland, OH, USA).

Tablet fabrication and dissolution

MA mixture with lactose

Lactose (494 mg), MA (100 mg) and magnesium stearate (6 mg) were mixed together in a 20-mL glass vial under nitrogen, overnight on 180° variable-speed flask shaker at 20 rev min^{-1} (St John Associates Inc., Beltsville, MD, USA).

Tablet fabrication

The above MA formulation with microgel was pressed into 60-mg tablets of 2 mm thickness and 7 mm diameter, using a hydraulic laboratory press (Catalogue No. 3850; Mini-C, Carver, Inc., Wabash, IN, USA), applying a force of 31 000 N at ambient temperature for 1 min. The same procedure was applied for both the MA formulation with Carbopol and the MA mixture with lactose. Friability of the tablets was tested using the procedure described in the USP monograph 1216 (USP 23, NF 18, The United States Pharmacopeial Convention, Inc. 1994, p. 1981). Briefly, 20 tablets were weighed (W1) and rotated for 100 revolutions in 4 min in a Roche friabilator. The tablets were then reweighed (W2) and the percentage friability (%F) was calculated as $\%F = [(W1 - W2/W1)]$. Friability results were in accordance with the pharmacopoeia limits ($F < 1\%$).

Media for dissolution

Aqueous solutions containing 1% SDS were used in dissolution tests as described in the USP monograph of MA (USP 23, NF 18, The United States Pharmacopeial Convention, Inc. 1994, p. 994). The following two solutions were prepared: (i) 1% SDS in 50 mM sodium phosphate buffer, pH 6.8 ± 0.1; and (ii) 1% aqueous SDS, pH 1.8 ± 0.1.

Each tablet was placed in a 50-mL conical vial (Ultident Scientific, St Laurent, PQ, Canada) containing 40 mL of the dissolution medium pre-equilibrated at 37°C. The vial was incubated at 37°C and shaken at 20 rev min^{-1} using a 180° variable-speed flask shaker. A liquid sample (1 mL) collected at a specified time was transferred into a 1.5-mL Eppendorf tube and centrifuged at 10 000 *g* for 15 min. The supernatant was then separated and diluted with the respective dissolution medium to a concentration of 3–50 μg mL^{-1}, and transferred to a 96-well quartz plate (Hellma GmbH & Co KG, Müllheim, Germany). The MA concentration in the samples was determined by measuring the optical density ($\lambda = 292$ nm) using a SpecraMax Plus384 plate reader (Molecular Devices, Sunnyvale, CA, USA) and comparing it with the optical density of standard solutions of MA in the same solvent. All dissolutions were performed three times and the results represent the mean ± s.d., $n = 3$.

Preparation of MA liquid dosage forms

Control

Appropriate amounts of MA were suspended in a 0.5 wt% aqueous solution of methylcellulose. The suspensions were equilibrated at room temperature by vortexing for 20 min. The final pH of the mixture was 6.6.

Pluronic formulations

Appropriate amounts of MA were suspended in an aqueous solution containing a mixture of 2% F127 and 0.4% L61. The mixture was vortexed at room temperature for 20 min. The final pH of the mixture was 6.4.

Formulations with microgel

Appropriate amounts of solid formulation were suspended in deionized water. The mixture was vortexed at room temperature for 20 min. The final pH of the mixture was 3.3.

Animal studies

All animal studies were conducted according to the Guidelines for Care and Use of Experimental Animals (US Department of Agriculture, February 15, 1991). Female Sprague–Dawley rats (225–250 g) were obtained from Charles River Canada Inc. (St Constant, PQ, Canada) and were kept three per cage with air filter cover

in a controlled environment (12-h light–dark cycle, lights on at 0600 h; temperature 22 ± 1°C). The animals had free access to Purina mouse chow (Pro Lab PMH 4018, Trademark of Agway, Syracuse, NY, USA) and water. The animals were randomly divided into groups of three. The MA formulations were administered orally by gavage. The dosing volume was 5 mL kg^{-1}. After administration, blood samples were collected at 0.5, 1, 3, 6, 10 and 24 h. The animals were anaesthetized by inhalation of isoflurane (Bimeta-MTC, Animal Health Inc., Cambridge, ON, Canada). The blood samples (approx. 450 μL) were collected from the jugular vein into heparinized tubes and immediately placed on ice. The plasma was separated from the blood cells by centrifugation at 2000 *g* for 5–10 min at 4°C. The plasma samples were frozen immediately on dry ice and stored at −80°C until assayed.

In microgel passage studies, adult pathogen-free male Harlan Sprague–Dawley rats (280–320 g) were acclimated to the environmentally controlled quarters (25 ± 1°C and 12-h light–dark cycle) for 5–6 days before the experiments. The animals had free access to the laboratory rodent diet and water until 18 h before being used in experiments, at which time food but not water was withdrawn. An appropriate sample of ^{14}C-labelled microgel suspension equilibrated at room temperature for 48 h under gentle agitation (3% w/w in water; pH 4.3; 0.25 g total) was administered by gavage to the middle of the oesophagus (Riley et al 2001b). The animals were kept in metabolism cages and were allowed free access to water until they were killed ($n = 3$) at pre-determined times (0.25, 1, 4, 6 h) by cervical dislocation after isofluorane anaesthesia. The entire gastrointestinal tract was rapidly removed and sectioned to determine the distribution of the labelled microgel. Samples of liver, kidneys, blood and urine were also collected.

For scintillation counting, the ex-vivo tissue samples were dissolved in Soluene-350 solubilization liquid. The resulting liquid samples were diluted 25-fold by a Hionic-Fluor scintillation fluid and the radioactivity was measured using a Tri-Carb 2100TR liquid scintillation analyser. All equipment and solvents used in the scintillation studies were obtained from Perkin Elmer, Inc. (Boston, MA, USA). Background counts were obtained for each tissue section and allowances for the background noise were made. Typically, the background noise was below 5% of the count in the tissue samples. The microgel fraction in the tissue was calculated as a sample count relative to the total count in all samples taken at a given time after administration (Riley et al 2001b). All measurements were taken in triplicate.

High-performance liquid chromatography analysis of MA in plasma

The MA concentration in plasma samples was measured using a reverse phase high-performance liquid chromatography procedure with UV detection based on that described by Gaver et al (1985) and Camaggi et al (1995). Briefly a 50:50 (v/v) hexane/ethyl acetate mixture (500 μL) was added to each of 100 μL of the plasma samples. The resulting mixtures were shaken by vortex for 5 min, centrifuged at 8000 *g* for 5 min, and the organic layer (400 μL) was separated and dried in vacuum. The residue was reconstituted with 250 μL of acetonitrile–water (1:1, v/v). The samples (100 μL each) were analysed using a Waters reverse phase high-performance liquid chromatography system, comprising a Model 1525 binary pump, a Model 2487 UV/vis absorbance detector, and a Model 2700 sample manager. Samples were injected into a C18 Luna column (250×4.6 mm, 5 μm; Phenomenex, Inc., CA, USA) and were analysed using isocratic conditions (0.1% trifluoroacetic acid in water to 0.1% trifluoroacetic acid in acetonitrile, 40:60 v/v, flow rate 1 mL min^{-1}). The column eluent was monitored at 288 nm. Calibration and quality control samples were prepared by spiking 100 μL of rat plasma with 10 μL MA solution in acetonitrile/water mixture (1:1, v/v) to give final drug concentrations over the range of 0–5000 ng mL^{-1}.

Pharmacokinetics

The area under the curve (AUC) of plasma concentration of MA versus time was calculated for each data set by the trapezoidal method from the point of dosing ($t = 0$) until the last quantified point ($t = 24$ h). Each data point of the MA plasma concentration was obtained as an average of the readings in samples from three animals, and the errors represent the s.e.m. The mean peak plasma concentration (C_{max}) was calculated as an average of the actual highest plasma concentration found in samples.

Statistical analysis

All experiments were performed in triplicate. Our earlier experience with oral drug bioavailability studies using rats demonstrated that improvement of the power of study by increasing the number of animals from three to six was very limited (data not presented). All statistical analyses were done using SPSS version 12.0 software (SPSS Inc., Chicago, IL, USA). In all analyses $P < 0.05$ indicated significance.

Statistical analysis of the effect of the Pluronic formulation versus the control formulation of MA (dose 1 mg kg^{-1}), and the effect of the microgel formulation versus the control formulation of MA (dose 10 mg kg^{-1}), on plasma concentration of MA at 0.5, 1, 3, 6, 10 and 24 h was performed using analysis of variance for repeated measures, and one-way analysis of variance at each time point. Statistical analysis of the effect of the Pluronic formulation versus the control formulation of MA at doses 1, 3, 5, 7 and 10 mg kg^{-1} on AUC was performed using two-way analysis of variance with treatment and dose as factors. Student's *t*-test was applied to compare AUC for control and Pluronic formulations of MA for each dose, and to compare C_{max} and AUC of MA for control and microgel formulations of MA. Statistical analysis of the effect of pH on the release of MA from microgel, and the effect of pH on the release of MA from Carbopol formulations at 1, 2, 3, 4, 5, 6, 7 and 8 h was

performed using Student's *t*-test for each time point. The differences of microgel fractions determined at all specified parts of the gastrointestinal tract and in different organs were compared using one-way analysis of variance separately for each passage time point (0.5, 1, 4 and 6 h).

Results and Discussion

Effect of Pluronic block copolymers on oral bioavailability of MA

Based on previously described results of P-gp modulation by Pluronics (Kabanov et al 2003), in the present study, we used the combination of Pluronic L61 and Pluronic F127. In the following set of experiments we evaluated plasma pharmacokinetics of MA administered by oral gavage to rats at the doses of 1, 3, 5, 7 and 10 mg kg^{-1}. The drug was administered in either non-formulated form as a suspension in 0.5% methylcellulose (control experiments), or formulated as a suspension with the composition containing 2% w/v Pluronic F127 and 0.4% w/v Pluronic L61. The results of these experiments are shown in Figure 1. The Pluronic formulation produced statistically significant 1.5- to 2-fold increases in AUC with the drug doses ranging from 1 to 7 mg kg^{-1} compared with the non-formulated drug. This enhanced effect became negligible at a dose of 10 mg kg^{-1}. In both the control experiments where animals were dosed with the non-formulated MA as well as in the group that received the Pluronic-based formulation, the dependence of AUC on the drug dose reached a plateau at the MA dose of 10 mg kg^{-1}, indicating that the presence of Pluronic did not provide any increase in the drug oral bioavailability at the highest dose tested. The results of these experiments demonstrate the absorption enhancement via the utilization of the Pluronic block copolymers in oral administration of MA when it is used in small to medium doses. The dose-dependent metabolic effects of MA are known (Canetta et al 1983; Von Roenn et al 1994; Engelson et al 1999) and in some cases the extended administration of high doses is desirable. The high doses of MA (up to 10 mg kg^{-1}) used for weight loss related indications (Tchekmedyian et al 1992) require further improvement of the formulation in order to bypass the plateau of the MA intestinal absorption at high doses. The latter is related to the transport mechanism with saturation kinetics (carrier transport). We hypothesized that by prolonging the residence time of MA we could enhance its bioavailability after oral administration. To this end, the Pluronic-based microgels were developed.

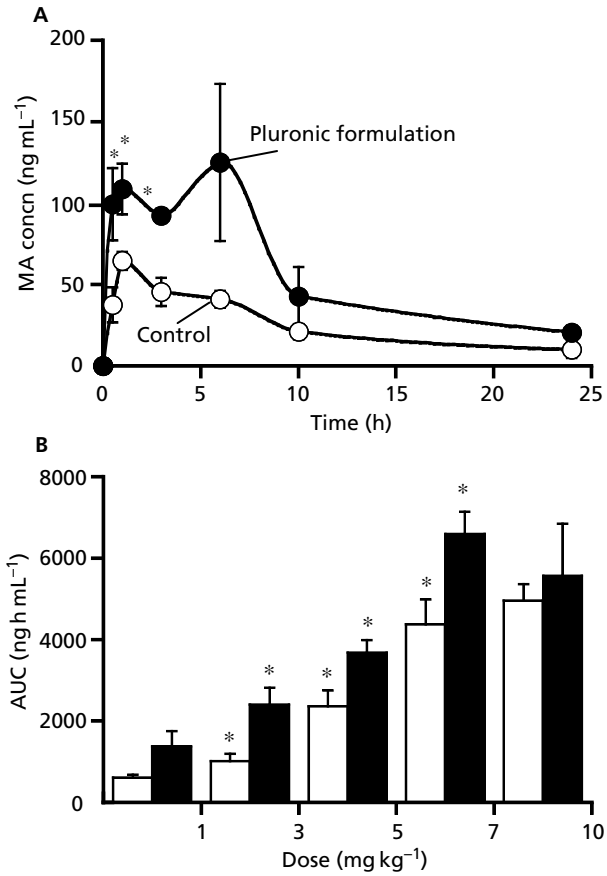

Figure 1 A. Typical kinetics of megestrol acetate (MA) concentration in plasma after oral administration of control and Pluronic formulations in a rat model. The control formulation comprised a MA suspension in a 0.5 wt% aqueous solution of methylcellulose; the Pluronic formulation comprised an aqueous solution of Pluronic F127 and L61 (2.4% total, F127:L61 5:1 w/w). The MA dose was 1 mg kg^{-1} by gavage. $*P < 0.05$, control versus Pluronic formulation. B. Area under the plasma concentration time curve (AUC) of MA administered orally by gavage in a rat model. Closed and open columns correspond to the MA administered in Pluronic and control formulations, respectively. $*P < 0.05$, control versus Pluronic formulation.

Controlled release of MA from Pluronic-based microgels

Having demonstrated the enhanced bioavailability with the liquid dosage forms of Pluronic copolymers, we proceeded to evaluate MA release from the Pluronic-based microgel formulations. Tablet dosage forms were tested in-vitro, and the MA release from the equilibrium-swollen microgels as well as their passage through the gastrointestinal tract were evaluated in a rat model.

In-vitro release

The swelling of the Pluronic-PAA microgel particles in aqueous media is profoundly pH-dependent, with both the rate of swelling and the extent of the equilibrium swelling increasing with the pH, especially above the pK_a of the carboxyls, which was found to be 6.27 for the L92-PAA microgels (Bromberg et al 2003). This pH sensitivity of the microgels loaded with drugs can be exploited in oral drug delivery since at low pH, such as in the stomach, these hydrogels remain collapsed, while the drastic pH change in the upper small intestine leads to dramatic swelling and thus the drug can be released. We tested the in-vitro release from the microgel-based tablets at pH 1.8

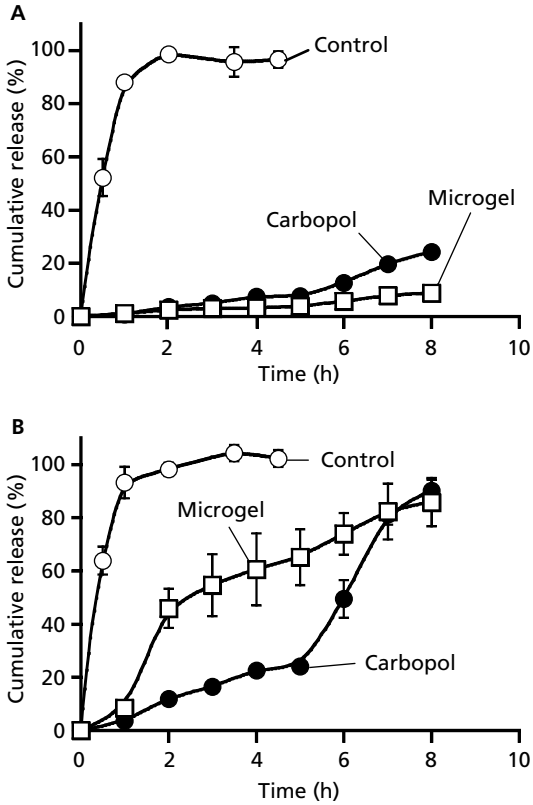

Figure 2 Kinetics of cumulative release of megestrol acetate at 37°C from the control, Carbopol, and microgel tablet dosage forms at pH 1.8 (A) and pH 6.8 (B) in the USP dissolution tests. $P < 0.05$ for all time points between 1 and 8 h (pH 1.8 compared with pH 6.8 for Carbopol tablets, and pH 1.8 compared with pH 6.8 for microgel tablets).

and pH 6.8, which modelled conditions in the stomach and intestine, respectively (Figure 2).

The MA release from the tablets was dramatically lowered in the presence of either Carbopol 974 or microgel. Whereas the pH did not really affect the release rate in the control formulations, the drug was essentially retained by Carbopol or the microgel at pH 1.8, and it was slowly released at pH 6.8, the conditions within the intestine. Relative to Carbopol 974, the microgel seemed to provide greater retention of the drug at acidic pH. This correlates well with the hydrophobic nature of the L92-PAA-ethylene glycol dimethacrylate microgels, containing about 36% of the water-insoluble poly(propylene oxide) per dry weight. The microgels require higher electrostatic repulsive energy for swelling and tablet disintegration as compared with Carbopol with its pK_a of 4.96. The microgels are more tightly cross-linked by the hydrophobic associations than Carbopol and hence have better drug retention at acidic pH.

Oral bioavailability of MA using microgel-based formulations

The microgel formulations of MA and the control formulation (MA suspension in 0.5% methylcellulose) were

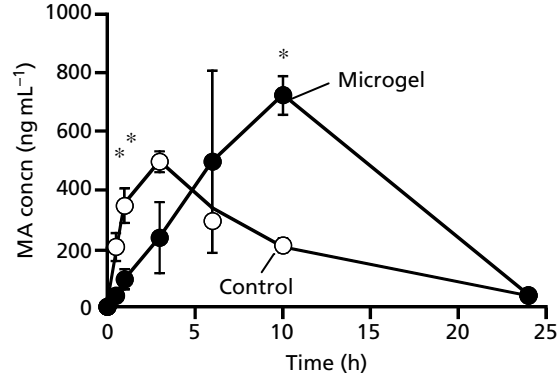

Figure 3 Kinetics of megestrol acetate (MA) concentration in plasma after oral administration of the control and microgel formulations in a rat model. The dose of MA was 10 mg kg^{-1} by gavage. The formulation comprised a 1 wt% aqueous suspension of the MA-loaded microgels. *$P < 0.05$, control versus microgel formulation.

administered orally by gavage to rats at a dose of 10 mg kg^{-1}. Figure 3 shows the MA plasma concentration versus time in rats dosed with the control and formulated samples. The C_{max} of MA in plasma determined for the rats dosed with the microgel formulation and for the control were 723 ± 66 ng mL^{-1} and 496 ± 36 ng mL^{-1}, respectively ($P = 0.04$), and the AUC values were 9261 ± 1451 ng h mL^{-1} and 4953 ± 402 ng h mL^{-1}, respectively ($P = 0.06$). The time to achieve C_{max} was 10 h for the group treated with the microgel formulation and only 3 h for the control group.

These results demonstrate a significant increase in bioavailability of the drug. Importantly, the time to reach C_{max} for the microgel-formulated MA was 3-fold longer than that of the non-formulated drug and Pluronic formulation, suggesting that microgel carrier indeed increased the drug residence time at the absorption site, probably owing to the enhanced retention of the microgel. In order to verify the latter hypothesis, we studied the passage of microgels through the gastrointestinal tract.

Passage of microgels through the gastrointestinal tract

Encouraged by the drug release results from microgel formulations, we proceeded to the in-vivo study of the microgel passage through the rat intestine. The study was designed to monitor the microgel gastrointestinal distribution profile at times ranging from 15 min to 6 h after administration. The pH of 4.3 of the administered microgel suspension was chosen because it allowed for a sufficiently flowable formulation and modelled a successful study of PAA transit through the gastrointestinal tract in rats (Riley et al 2001b). The results of the passage study are shown in Figure 4. Even at 15 min after administration, no significant presence of the microgel was observed in the oesophagus, with the most significant microgel fraction retained in the stomach.

The progress of the microgel transit can be seen by comparing the microgel distributions over time (Figure 4).

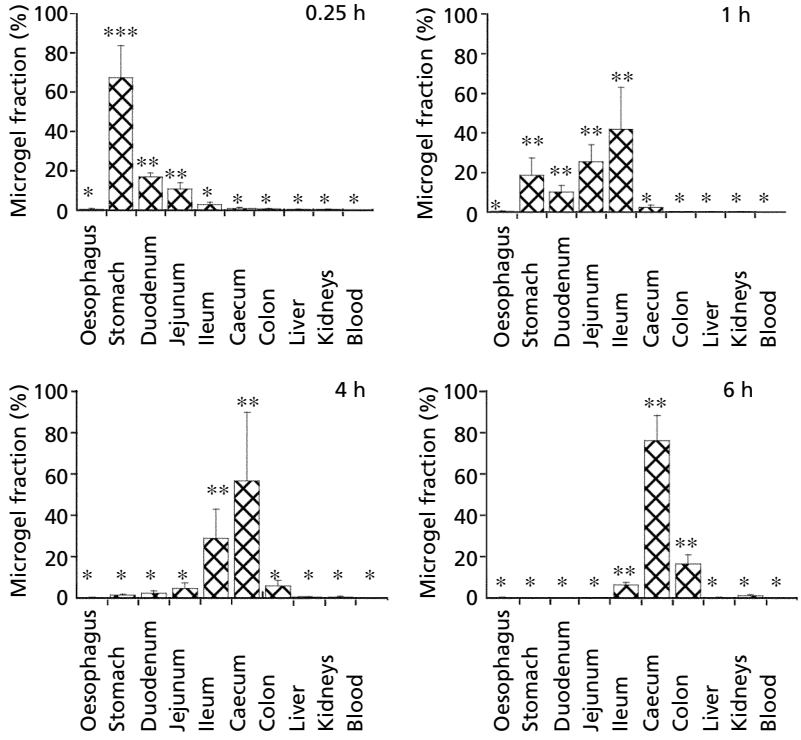

Figure 4 Microgel passage in the rat intestine (n = 3; bars represent the s.d). The L92-PAA-ethylene glycol dimethacrylate microgels were administered into the rat oesophagus at pH 4.3 and t = 0. Asterisks indicate statistically different groups ($P < 0.001$).

At 1 h, significant fractions of the microgel were observed in the stomach as well as in the lower parts of the small intestine, whereas after 4 h, the largest fraction of the microgel appeared in the large intestine. After 6 h, all of the microgel fractions were concentrated in the lower gastrointestinal tract. A similar transit profile has been observed with ultra-high molecular weight PAA fractions (MW > 3 MDa) in a rat model (Riley et al 2001b). Faeces, randomly collected after 6 h, contained 12–16% of the microgel relative to the total in the gastrointestinal tract measured at 6 h. Importantly, no significant fractions of the labelled microgels were found in liver, kidney or blood samples, which proves the absence of any systemic absorption.

Conclusions

We studied the effect of several Pluronic-based formulations on the release and bioavailability of MA in-vitro and in a rat model. The Pluronic copolymers possess surfactant properties and self-assemble into micelles in aqueous media. The micelles solubilize hydrophobic drugs such as MA. It was demonstrated that an aqueous formulation comprising a mixture of hydrophobic (L61) and hydrophilic (F127) Pluronic copolymers significantly enhanced bioavailability of orally administered MA at doses up to 7 mg kg^{-1}. The enhancement effect plateaued at a MA dose of 10 mg kg^{-1}. In the present study, we used novel Pluronic-based microgels as drug vehicles in oral administration. The microgels (spherical gel particles of sub-millimetre size) comprise a cross-linked network of PAA onto which the Pluronic chains are covalently attached. The feasibility of microgels based on hydrophobic Pluronic L92 and PAA in tablet preparations was demonstrated. The tablets exhibited dramatically lower MA initial burst release compared with the control (lactose-based dosage form). Moreover, the MA release was pH-dependent, with the drug retained by the microgel at pH 1.8 and released slowly at pH 6.8. The microgel particles provided a superior drug retention capability at acidic pH when compared with Carbopol 974. In the rat model, a significant increase of MA bioavailability was observed when the microgel-formulated MA was administered orally, as a result of the enhanced retention of the microgel. The study of the microgel passage through the gastrointestinal tract proved both the microgel retention characteristic of a very high molecular weight polymer and the absence of any systemic absorption of the polymer.

References

Alakhov, V. Y., Moskaleva, E. Y., Batrakova, E. V., Kabanov, A. V. (1996) Hypersensitization of multidrug resistant human ovarian carcinoma cells by pluronic P85 block copolymer. *Bioconjug. Chem.* **7**: 209–216

Alakhov, V. Y., Klinski, E., Li, S., Pietrzynski, G., Venne, A., Batrakova, E., Bronich, T., Kabanov, A. (1999) Block copolymer-based formulation of doxorubicin. From cell screen to clinical trials. *Colloids Surf. B, Biointerfaces* **16**: 113–134

Allemann, E., Leroux, J., Gurny, R. (1998) Polymeric nano- and microparticles for the oral delivery of peptides and peptidomimetics. *Adv. Drug Deliv. Rev.* **34**: 171–189

Batrakova, E. V., Li, S., Alakhov, V. Y., Elmquist, W. F., Miller, D. W., Kabanov, A. V. (2003a) Sensitization of cells overexpressing multidrug-resistant proteins by pluronic P85. *Pharm. Res.* **20**: 1581–1590

Batrakova, E. V., Li, S., Alakhov, V. Y., Miller, D. W., Kabanov, A. V. (2003b) Optimal structure requirements for pluronic block copolymers in modifying P-glycoprotein drug efflux transporter activity in bovine brain microvessel endothelial cells. *J. Pharmacol. Exp. Ther.* **304**: 845–854

Bromberg, L., Temchenko, M., Hatton, T. A. (2002) Dually responsive microgels from polyether-modified poly(acrylic acid): swelling and drug loading. *Langmuir* **18**: 4944–4952

Bromberg, L., Temchenko, M., Hatton, T. A. (2003) Smart microgel studies. Polyelectrolyte and drug-absorbing properties of microgels from polyether-modified poly(acrylic acid). *Langmuir* **19**: 8675–8684

Bussemer, T., Otto, I., Bodmeier, R. (2001) Pulsatile drug-delivery systems. *Crit. Rev. Ther. Drug Carrier Syst.* **18**: 433–458

Camaggi, C. M., Strocchi, E., Martoni, A., Zamagni, C., Cacciari, N., Robustelli della Cuna, G., Pavesi, L., Tedeschi, M., Silva, A., Pannuti, F. (1995) Pharmacokinetic evaluation of two different formulations of megestrol acetate in patients with advanced malignancies. *Cancer Chemother. Pharmacol.* **36**: 356–359

Canetta, R., Florentine, S., Hunter, H., Lenaz, L. (1983) Megestrol acetate. *Cancer Treat. Rev.* **10**: 141–157

Chan, L. M., Lowes, S., Hirst, B. H. (2004) The ABCs of drug transport in intestine and liver: efflux proteins limiting drug absorption and bioavailability. *Eur. J. Pharm. Sci.* **21**: 25–51

Danson, S., Ferry, D., Alakhov, V., Margison, J., Kerr, D., Jowle, D., Brampton, M., Halbert, G., Ranson, M. (2004) Phase I dose escalation and pharmacokinetic study of pluronic polymer-bound doxorubicin (SP1049C) in patients with advanced cancer. *Br. J. Cancer* In press

Engelson, E. S., Pi-Sunyer, F. X., Kotler, D. P. (1999) Effects of megestrol acetate and testosterone on body composition in castrated male Sprague-Dawley rats. *Nutrition* **15**: 465–473

Eltabbakh, G. H., Moody, J., Garafano, L. L., Hammond, J. M. (1999) The combination paclitaxel, carboplatin and megestrol acetate is effective in women with recurrent uterine papillary serous adenocarcinoma. *Eur. J. Gynaecol. Oncol.* **20**: 18–19

Farinha, A., Bica, A., Tavares, P. (2000) Improved bioavailability of a micronized megestrol acetate tablet formulation in humans. *Drug Dev. Ind. Pharm.* **26**: 567–570

Fleming, G. F., Amato, J. M., Agresti, M., Safa, A. R. (1992) Megestrol acetate reverses multidrug resistance and interacts with P-glycoprotein. *Cancer Chemother. Pharmacol.* **29**: 445–449

Gaver, R. C., Pittman, K. A., Reilly, C. M., Smyth, R. D., Goodson, P. J., Fenzl, E., Breault, G. O. (1985) Bioequivalence evaluation of new megestrol acetate formulations in humans. *Semin. Oncol.* **12**: 17–19

Gershanik, T., Benita, S. (2000) Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. *Eur. J. Pharm. Biopharm.* **50**: 179–188

Hurter, P. N., Alexandridis, P., Hatton, T. A. (1995) Solubilization in amphiphilic copolymer solutions. *Surfactant Sci. Ser.* **55**: 191–235

Kabanov, A. V., Batrakova, E. V., Alakhov, V. Y. (2003) An essential relationship between ATP depletion and chemosensitizing activity of Pluronic block copolymers. *J. Control Release* **91**: 75–83

Kimura, Y., Aoki, J., Kohno, M., Ooka, H., Tsuruo, T., Nakanishi, O. (2002) P-glycoprotein inhibition by the multidrug resistance-reversing agent MS-209 enhances bioavailability and antitumor efficacy of orally administered paclitaxel. *Cancer Chemother. Pharmacol.* **49**: 322–328

Kreuter, J. (1996) Nanoparticles and microparticles for drug and vaccine delivery. *J. Anat.* **189**: 503–505

Kruijtzer, C. M., Beijnen, J. H., Schellens, J. H. (2002) Improvement of oral drug treatment by temporary inhibition of drug transporters and/or cytochrome P450 in the gastrointestinal tract and liver: an overview. *Oncologist* **7**: 516–530

Kruijtzer, C. M., Boot, H., Beijnen, J. H., Lochs, H. L., Parnis, F. X., Planting, A. S., Pelgrims, J. M., Williams, R., Mathot, R. A., Rosing, H., Schot, M. E., Van Tinteren, H., Schellens, J. H. (2003) Weekly oral paclitaxel as first-line treatment in patients with advanced gastric cancer. *Ann. Oncol.* **14**: 197–204

Lemieux, P., Guerin, N., Paradis, G., Proulx, R., Chistyakova, L., Kabanov, A., Alakhov, V. A. (2000) Combination of poloxamers increases gene expression of plasmid DNA in skeletal muscle. *Gene Ther.* **7**: 986–991

Lum, B. L., Fisher, G. A., Brophy, N. A., Yahanda, A. M., Adler, K. M., Kaubisch, S., Halsey J., Sikic, B. I. (1993) Clinical trials of modulation of multidrug resistance. *Cancer* **72**: 3502–3514

Mahato, R. I., Narang, A. S., Thoma, L., Miller, D. D. (2003) Emerging trends in oral delivery of peptide and protein drugs. *Crit. Rev. Ther. Drug Carrier Syst.* **20**: 153–214

Markman, M., Kennedy, A., Webster, K., Kulp, B., Peterson, G., Belinson, J. (2000) Phase I trial of paclitaxel plus megestrol acetate in patients with paclitaxel-refractory ovarian cancer. *Clin. Cancer Res.* **6**: 4201–4204

Martindale, W., Reynolds, J. E. F. (1993) *The Extra Pharmacopoeia*. Pharmaceutical Press, London

Merisko-Liversidge, E., Liversidge, G. G., Cooper, E. R. (2003) Nanosizing: a formulation approach for poorly-water-soluble compounds. *Eur. J. Pharm. Sci.* **18**: 113–120

Muller, R. H., Jacobs, C., Kayser, O. (2001) Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. *Adv. Drug Deliv. Rev.* **47**: 3–19

Panasci, L., Jean-Claude, B. J., Vasilescu, D., Mustafa, A., Damian, S., Damian, Z., Georges, E., Liu, Z., Batist, G., Leyland-Jones, B. (1996) Sensitization to doxorubicin resistance in breast cancer cell lines by tamoxifen and megestrol acetate. *Biochem. Pharmacol.* **52**: 1097–1102

Raney, M. S., Anding, R., Fay, V., Polk, G. (2000) A pilot study to assess the use of megesterol acetate to promote weight gain in frail elderly persons residing in long-term care. *J. Am. Med. Dir. Assoc.* **1**: 154–158

Riley, R. G., Smart, J., Tsibouklis, D. J., Davis, J. A., Dettmar, P. W., Wilber, W. R. (2001a) The synthesis of radiolabelled analogues of the carbomers: ^{14}C-labelled poly(acrylic acid)s. *J. Biomed. Mater. Res. (Appl. Biomater.)* **58**: 102–107

Riley, R. G., Greena, K. L., Smart, J. D., Tsibouklis, J., Davis, J., Hampson, A. F., Dettmar, P. W., Wilber, W. R. (2001b) The gastrointestinal transit profile of ^{14}C-labelled poly(acrylic acids): an in vivo study. *Biomaterials* **22**: 1861–1867

Sethia, S., Squillante, E. (2003) Solid dispersions: revival with greater possibilities and applications in oral drug delivery. *Crit. Rev. Ther. Drug Carrier Syst.* **20**: 215–247

Sood, A., Panchagnula, R. (2001) Peroral route: an opportunity for protein and peptide drug delivery. *Chem. Rev.* **101**: 3275–3304

Tansan, S., Koc, Y., Aydin, H., Urbano, G., McCaffrey, R. (1997) Augmentation of vincristine cytotoxicity by megestrol acetate. *Cancer Chemother. Pharmacol.* **39**: 333–340

Tchekmedyian, N. S., Hickman, M., Heber, D. (1991) Treatment of anorexia and weight loss with megestrol acetate in patients with anorexia and AIDS. *Semin. Oncol.* **18**: 35–42

Tchekmedyian, N. S., Hickman, M., Siau, J., Greco, F. A., Keller, J., Browder, H., Aisner, J. (1992) Megestrol acetate in cancer anorexia and weight loss *Cancer* **69**: 1268–1274

Vasir, J. K., Tambwekar, K., Garg, S. (2003) Bioadhesive microspheres as a controlled drug delivery system. *Int. J. Pharm.* **255**: 13–32

Venne, A., Li, S., Mandeville, R., Kabanov, A. V., Alakhov, V. Y. (1996) Hypersensitizing effect of pluronic L61 on cytotoxic activity, transport, and subcellular distribution of doxorubicin in multiple drug-resistant cells. *Cancer Res.* **56**: 3626–3629

Verma, R. K., Krishna, D. M., Garg, S. (2002) Formulation aspects in the development of osmotically controlled oral drug delivery systems. *J. Control. Release* **79**: 7–27

Von Roenn, J. H., Murphy, R., Weber, K. M., Williams, L. M., Weitzmen, S. A. (1988) Megestrol acetate for treatment of cachexia associated with HIV infection. *Ann. Intern. Med.* **109**: 840–841

Von Roenn, J. H., Armstrong, D., Kotler, D. P., Cohn, D. L., Klimas, N. G., Tchekmedyian, N. S., Cone, L., Brennan, P. J., Weitzman, S. A. (1994) Megestrol acetate in patients with AIDS-related cachexia. *Ann. Intern. Med.* **121**: 393–399

Wang, L., Yang, C-P. H., Horwitz, S. B., Trail, P. A., Casazza, A. M. (1994) Reversal of the human and murine multidrug-resistance phenotype with megestrol acetate. *Cancer Chemother. Pharmacol.* **34**: 96–102

Yang, C. -P. H., DePinho, S. G., Greenberger, L. M., Arceci, R. J., Horwitz, S. B. (1989) Progesterone interacts with P-glycoprotein in multidrug-resistant cells and in the endometrium of gravid uterus. *J. Biol. Chem.* **264**: 782–788