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Oral Administration of Chemotherapeutic Agents Using Complexation Hydrogels

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ABSTRACT

Carriers were synthesized to target delivery of a chemotherapeutic agent, bleomycin, to the upper small intestine in response to the pH shift when entering the upper small intestine from the stomach. Complexation hydrogels capable of pH-responsive swelling were used to form these carriers. Hydrogel nanospheres composed of methacrylic acid (MAA) and poly(ethylene glycol) (PEG) were loaded with bleomycin. Loading of bleomycin was performed by *in situ* polymerization and release of bleomycin from the nanospheres was measured by UV spectrophotometry. Results showed that bleomycin release from the nanospheres was responsive to the pH of the environment surrounding the nanospheres. In addition to pH-responsive release of bleomycin, the hydrogel nanospheres are also able to enhance the permeability of an *in vitro* model of the intestinal epithelium. Increasing the permeability of the intestinal epithelium could aid in transport of bleomycin from the lumen of the small intestine out into the bloodstream.

INTRODUCTION

There are numerous potential advantages that oral administration of chemotherapeutic agents has over other methods of administration such as injection [1]. Some of these advantages include increased efficacy, lower toxicity, increased flexibility of dosing schedule and higher patient comfort. Recent studies comparing oral administration of a variety of different chemotherapeutic agents to intravenous administration found decreased toxicity and comparable, if not improved, efficacy [2-4]. The harsh environment of the gastrointestinal tract and the potential toxicity of chemotherapeutic agents requires that our carriers selectively delivery the drugs to a site promising for absorption into the bloodstream with low toxicity. During the development of these carriers, studies have focused on the use of a single chemotherapeutic agent, bleomycin. Bleomycin is a glycopeptidic antibiotic used in chemotherapy for approximately 30 years [5]. In the future, other chemotherapeutic agents will be studied to expand the potential uses for these carriers to include a variety of drugs from different classes of chemotherapeutic agents.

Successful development of the carriers requires fulfillment of a number of requirements. A delivery vehicle capable of being loaded with bleomycin and subsequently releasing it, with attention focused to the efficiency of each process, must first be developed. The pH increase that occurs when passing from the stomach to the upper small intestine is the trigger to initiate release so this carrier must be able to selectively release bleomycin in response to a pH shift used to simulate this physiological event. The kinetics of release from the carrier must be appropriate given the projected residence time for the carrier particles in the region of the digestive tract most suitable for absorption of bleomycin. The final property of our carriers to be analyzed is

the ability to enhance the permeability of an *in vitro* model of the intestinal epithelium as this should aid transport of bleomycin out of the intestinal lumen.

EXPERIMENTAL DETAILS

Nanosphere synthesis

Hydrogel nanospheres were formed from a diluted monomer mixture by UV-initiated free radical polymerization. The monomer mixture was composed of MAA (Polysciences, Warrington, PA), poly(ethylene glycol) monomethylether monomethacrylate (molecular weight 1000) (PEGMA) (Polysciences), tetraethylene glycol dimethacrylate (TEGDMA) (Polysciences) and Irgacure 184® (1-hydroxy-cyclohexl-phenylketone (HCPK)) (CIBA-GEIGY, Hawthorne, NY). Prior to use in the reaction, MAA was vacuum distilled at 54 °C and 25 mm Hg to remove the hydroquinone that was used as an inhibitor.

The monomer solution has a 1:1 ratio of MAA to ethylene glycol units. Because the PEGMA has approximately 23 ethylene glycol units to give it a molecular weight of 1000, the molar ratio of PEGMA to MAA is therefore 1:23. A total weight of 2.0 g of PEGMA and 3.6 g of MAA yields the desired ratio and a total of 0.043 moles. For a typical reaction, a TEGDMA concentration of 1.5 mol% was added to the monomer mixture and 0.1 wt% HCPK was added to initiate the reaction.

This concentrated monomer solution was diluted in deionized water to create the working solution that was used to form the hydrogel. The working solution contained 800 µL of concentrated monomer solution for every 100 mL of deionized water. Because oxygen will inhibit the free radical-initiated polymerization, the polymerization was carried out in an oxygen-free environment. To create these conditions, the working solution was sealed with a rubber stopper in an erlenmeyer flask and nitrogen was bubbled through the solution for 30 minutes. The sealed flask was then exposed to a UV lamp (Efos Acticure™ Ultraviolet/Visible spot cure system, Mississauga, Ontario) providing an intensity of 45 mW/cm^2 for 15 minutes.
The polymerization yielded a suspension containing hydrogel nanospheres. This nanosphere suspension was then washed where noted. The washing procedure involved placing the nanosphere suspension in a cellulose dialysis membrane with a molecular weight cut-off of 25,000 (Spectra Por® 7, Spectrum Laboratories Inc., Rancho Dominguez, CA) and then placing this membrane in a low pH (pH = 2.0) bath. The washing duration was typically 10 min.

After the wash was completed, the suspension was removed from the dialysis membrane, placed in a 50 mL conical tube and frozen for at least 24 h at –20 °C. To obtain the hydrogel nanospheres, the frozen solution was lyophilized at –50 °C under vacuum (Labconco Model 77500, Kansas City, MO) until only the dry nanospheres remained.

Bleomycin loading

Bleomycin loading into the hydrogel carriers was achieved through *in situ* polymerization. Bleomycin was dissolved in the deionized water used to dilute the concentrated monomer solution prior to nitrogen purge at concentrations ranging from 0.01 – 0.05 mg/mL. As the poly(methacrylic acid) grafted with poly(ethylene glycol) (P(MAA-g-EG)) nanospheres form in the monomer solution, some of the bleomycin becomes trapped within the nanospheres.

The efficiency of bleomycin loading was measured by UV spectrophotometry (Perkin Elmer Model Lambda 10, Norwalk, CT). Bleomycin was diluted at various concentrations ranging from 0.001 to 0.1 mg/mL and the relation between the absorbances of these solutions at 300 nm and the bleomycin concentration was determined. Using the intensity of the absorbance at 300 nm, the amount of bleomycin incorporated into the nanospheres during polymerization was measured.

Before placing the samples in the UV spectrophotometer, the samples were run through a filter using a syringe (Monoject®, Sherwood Medical, St Louis, MO) attached to a 0.2 μm nylon filter. Because the absorbance for bleomycin is in the UV range, quartz cuvettes were used with a sample volume of 2mL added to the cuvette. Samples are scanned over a range of wavelengths and the intensity at 300 nm is used to determine the bleomycin concentration present.

Release studies

Release experiments were performed with a dissolution apparatus (Distek model 2100B, North Brunswick, NJ). Bleomycin-loaded nanospheres were added to a vessel containing 100 mL of solution stirred at 100 rpm and maintained at 37 °C. For release studies done at a constant pH, 3 mL samples were taken from the release vessel, filtered through the 0.2 μm filter and stored for later analysis by UV spectrophotometry. The 3 mL of solution removed was replaced with solution of the appropriate pH and this loss of initial solution is accounted for in later analysis. Studies done at high pH were done in a phosphate buffered saline (PBS) solution at a pH of 7.4. Both studies were carried out for a duration of 90 minutes.

To better simulate the environment of the gastrointestinal tract, some studies were done with a pH shift from low to high to better mimic the passage from the stomach into the duodenum. For these studies, the initial solution was again hydrochloric acid diluted in water to a pH of 2.0. After a period of 60 minutes, 5M NaOH and PBS were added to raise the pH to 7.0 and samples were taken for an additional 120 minutes. The necessary volume of NaOH and PBS to raise the pH of the vessel from 2.0 to 7.0 was determined prior to the release experiment with the same solutions to be used in the release experiment.

The time points at which samples were taken were close together at the beginning of the experiment and again after the pH increase to measure the release initially and after nanosphere swelling. As with the constant pH release experiments, the absorbance of all samples at 300 nm was measured by UV spectrophotometry to measure the bleomycin concentration in the release vessel.

Transepithelial electrical resistance (TEER) measurements

Caco-2 cells (human colon adenocarcinoma) were seeded on Transwell® plates at low density and allowed to grow and differentiate over the course of 21-24 days. Hank's Balanced Salt Solution (HBSS) (Hyclone South Plainfield, NJ) was added to the cells 1 hour before the TEER measurements are taken to allow the cells to equilibrate. P(MAA-g-EG) nanospheres, with no bleomycin loaded, were added to the apical side of the well at different concentrations ranging from 5-20 mg/mL and TEER measurements were taken with a 2 prong electrode (EVOM, World Precision Instruments, Sarasota, FL) over the course of 2 hours. While the measurements were being taken, the cells were placed on a heating mat to maintain the

temperature at 37 °C as the resistance value is exponentially related to the temperature. Control cells remained in HBSS without any nanospheres.

RESULTS

Nanosphere synthesis and bleomycin loading

Formation of the nanospheres was dependent on the ratios of the components used in the monomer mixture, the exposure time to the UV light and the conditions of the low pH wash. Increasing the amount of crosslinking agent and extending the duration of either the UV exposure or the low pH wash led to agglomeration of the nanospheres rather than formation of a homogeneous suspension. Dissolution of bleomycin within the concentration range given did not inhibit the formation of nanospheres. The loading efficiency of the *in situ* polymerization was found to be 76% (+/- 9% n=3).

Release studies

Release studies done at a constant pH showed more release of bleomycin from nanospheres at high pH. These results were anticipated, as bleomycin should pass more readily from the nanospheres in a swollen state. The maximal release occurred after 90 minutes for both pH values with the release at pH 2.0 only 45% of that observed at pH 7.4 (M_t/M_∞ = 0.442 +/- 0.115 n=3). These experiments were performed with nanospheres that had not been washed at a low pH prior to lyophilization. The crosslinking density of these nanospheres was also lower than that given in the nanosphere synthesis protocol as only 0.75 mol% TEGDMA was used. Changes to the nanosphere synthesis protocol, matching the conditions listed above, were made to reduce the amount of bleomycin released at low pH and improve the release kinetics at the high pH.

Experiments where the pH was shifted during the release experiment were used to better simulate the *in vivo* environment when passing from the stomach into the small intestine. Results showed some release occurring at the low pH used to simulate the gastric pH with the remaining bleomycin released after the pH was raised. The results of the bleomycin release studies done with the pH change are shown in Figure 1. Release of bleomycin at pH 2.0 quickly reached values of approximately 25% of the total release with a plateau established before the pH increase (M_t/M_∞ at 60 min = 0.268 +/- 0.030 n=3). The residence time in the upper small intestine was estimated as 2 hours and release of bleomycin was controlled over this duration.

TEER studies

P(MAA-g-EG) nanospheres were added to the apical side of a Caco-2 monolayer with the concentration ranging from 5-20 mg/mL. The effect of the nanospheres on the electrical resistance across the Caco-2 monolayer was measured for a duration of 2 hours. Initial studies have shown a decrease in resistance, indicating an increase in permeability, for those cells treated with the nanospheres relative to control cells. The results of these studies are shown in Figure 2. The largest decrease in resistance was seen for the highest concentration of nanospheres, 20 mg/mL, with a maximal decrease of 55% relative to the control cells.

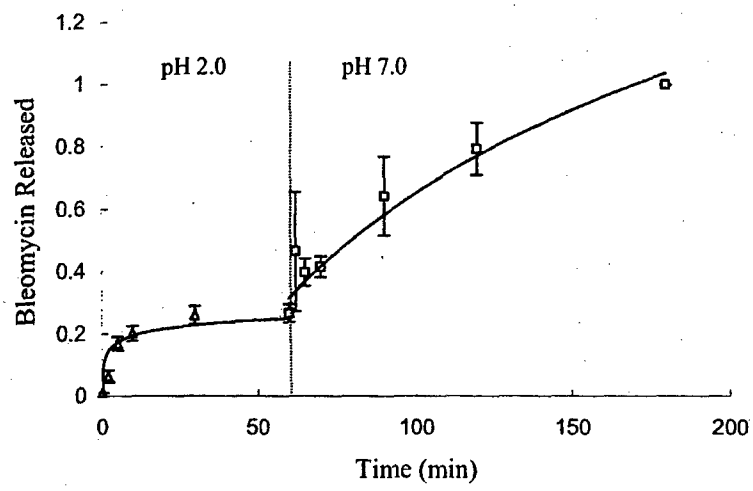

Figure 1: Bleomycin release, expressed as M_t/M_∞, over three hours in a release vessel. The pH of the vessel was changed from 2.0 to 7.0 after 60 minutes to simulate passage from the stomach into the small intestine.

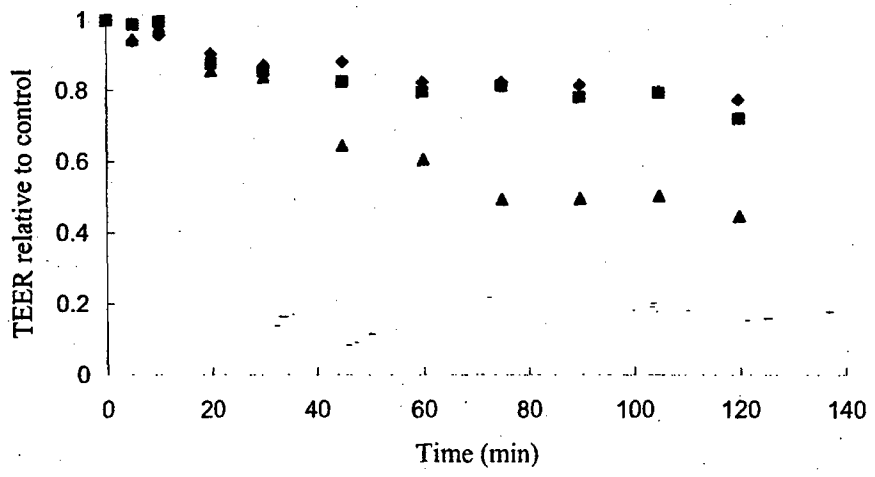

Figure 2: The value of the transepithelial electrical resistance of Caco-2 monolayers over two hours. Three different concentrations of P(MAA-g-EG) nanospheres were used, 5mg/mL (diamond), 10 mg/mL (square) and 20 mg/mL (triangle). The TEER value is given relative to the value for control cells at the same time.

CONCLUSIONS

The studies completed show the ability to form P(MAA-g-EG) nanospheres in the presence of bleomycin resulting in loading of bleomycin by *in situ* polymerization. The pH-responsive decomplexation and swelling of these nanospheres can be used to selectively release bleomycin in response to a pH shift similar to that seen when passing from the stomach to the upper small intestine. The nanospheres are also able to increase the permeability of an *in vitro* model of the intestinal epithelium, which could aid in transport of bleomycin out of the intestinal lumen and therefore increase the bioavailability.

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