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The effect of complexation hydrogels on insulin transport in intestinal epithelial cell models

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ABSTRACT

A novel class of pH-sensitive complexation hydrogels composed of methacrylic acid and functionalized poly(ethylene glycol) (PEG) tethers, referred to as P(MAA-*g*-EG) WGA, was investigated as an oral protein delivery system. The PEG tethers were functionalized with wheatgerm agglutinin (WGA), a lectin that can bind to carbohydrates in the intestinal mucosa, to improve residence time of the carrier and absorption of the drug at the delivery site. The ability of P(MAA-*g*-EG) WGA to improve insulin absorption was observed in two different intestinal epithelial models. In Caco-2 cells P(MAA-*g*-EG) WGA improved insulin permeability 9-fold as compared with an insulin only solution, which was similar to the improvement by P(MAA-*g*-EG). P(MAA-*g*-EG) and P(MAA-*g*-EG) WGA were also evaluated in a mucus-secreting culture that contained Caco-2 and HT29–MTX cells. Insulin permeability was increased 5-fold in the presence of P(MAA-*g*-EG) and P(MAA-*g*-EG) WGA. Overall, it is clear that P(MAA-*g*-EG) WGA enhances insulin absorption and holds great promise as an oral insulin delivery system.

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1. Introduction

Current estimates indicate that the cost of bringing a drug to market is nearing $1 billion dollars [1]. With this in mind, it is clear that in vitro tools that can screen and accurately predict the potential success of a drug candidate are necessary. Cell culture models to predict drug permeability are becoming a routine part of drug development within the pharmaceutical industry. While cell culture studies to predict drug absorption often take a minimum of 3 weeks, in vitro experiments have proven to be a more cost-effective method for initial permeability screening than in vivo studies.

The best established and accepted cell culture model for predicting drug absorption uses human colon adenocarcinoma cells (Caco-2) [2,3]. Previous research with this cell line has shown that studies are reproducible and correlate well with in vivo data [4]. Caco-2 cell monolayers are similar to the small intestine epithelial layer in that they differentiate into columnar absorptive cells and form a polarized monolayer that includes tight junctions. In addition, Caco-2 cells display a brush border, excrete typical brush border enzymes and express many carrier-mediated transport systems [2,5]. However, Caco-2 cells only differentiate into absorptive enterocytes, whereas the intestinal epithelial layer consists of a variety of different cell types, including goblet cells (mucus-secreting), enteroendocrine cells and M-cells. Also, due to the lack of goblet cells, there is no mucus layer lining the cellular monolayer.

Hilgendorf et al. [6] noted that a Caco-2 cellular monolayers form tight junctions which more closely represent the tightness of the junctions present in the colon, as opposed to the looser junctions present in the small intestine. The disadvantage of this is that permeability will be decreased for compounds which are transported predominantly by the paracellular mechanism. It has been shown that the permeability of paracellular markers using Caco-2 cells can be 100 times lower than the permeability in human small intestine [7].

For these reasons, researchers have investigated a variety of different cell lines to assess drug permeability. Some of the more common cell lines include MDCK (dog kidney epithelial cells), LLC-PK1 (pig kidney epithelial cells) and TC-7 (a subclone of Caco-2) [1,8]. While all of these cell lines have similar properties and certain advantages and disadvantages over the Caco-2 cell line, they all lack a mucus-producing cell.

The HT29 cell line is a human colon carcinoma cell line that contains both mucus and columnar absorptive cells [9]. Researchers have developed various subclones of this cell line that differentiate into predominantly mucus-secreting cells. Many of the HT29 subclones have been used in co-culture with Caco-2 cells and also by themselves to design a model that more accurately mimics the small intestinal epithelial layer [7,8,10–13].

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Our laboratory has successfully developed a class of environmentally sensitive complexation hydrogels containing methacrylic acid (MAA) and poly(ethylene glycol) (PEG) tethers [designated P(MAA-*g*-EG)] [14,15]. More specifically, P(MAA-*g*-EG) is a pH-responsive hydrogel that is capable of swelling and deswelling due to the formation of temporary physical cross-links, or interpolymer complexes, between the PMAA pendant groups and the tethered PEG chains. These systems can therefore utilize the pH shift between the stomach and the small intestine (from ~pH 2 to pH7) as an environmental trigger to deliver protein to the targeted site of delivery, which is the small intestine. Furthermore, the PEG tethers were functionalized with wheatgerm agglutinin (WGA), a lectin that can bind to carbohydrates in the intestinal mucosa, to improve residence time of the carrier and absorption of the drug at the delivery site. In this research we seek to evaluate insulin transport in the presence of functionalized complexation hydrogels across a Caco-2 cell monolayer and a Caco-2/HT29–MTX monolayer to determine differences in permeability.

2. Materials and methods

2.1. Hydrogel synthesis and functionalization

Hydrogel microparticles with a diameter of 90–150 μm were prepared by UV-initiated free radical solution polymerization as previously described [16]. P(MAA-*g*-EG) was functionalized with biotinylated WGA (B-WGA) through a biotin–avidin linkage as previously described [17]. Prior to polymerization the PEG chains were functionalized with biotin by established protocols [18,19] to allow for the addition of B-WGA by use of an avidin linker.

Microparticles containing PEG–biotin tethers were added to phosphate-buffered saline (PBS), pH 7.4. Avidin D (Vector Laboratories, Burlingame, CA) was added in a 1:1 M ratio to biotin. The solution was stirred for 30 min, after which the particles were filtered and washed. Particles were then resuspended in PBS, pH 7.4, and B-WGA (Vector Laboratories) was added in a 1.5:1 M ratio of B-WGA to avidin. After 1 h agitation, particles were washed and filtered. Particles were then lyophilized and stored at −20 °C until use. Functionalization was confirmed via HPLC (Waters 2695 Separations Module, Milford, MA).

2.2. General cell culture

Caco-2 cells were obtained from the American Type Culture Collection (Rockwell, MD) and HT29–MTX cells were a kind gift from Dr. Thecla Lesuffleur (INSERM, Paris, France). HT29–MTX cells are a sub-population of HT29 cells that were adapted to 10^{-6} M methotrexate (MTX) [9,20]. All cell types were cultured in Dulbecco's modified Eagle's medium (DMEM) (Mediatech, Herndon, VA) supplemented with 10% heat-inactivated fetal bovine serum (Cambrex, East Rutherford, NJ), 1% non-essential amino acids (Mediatech), 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (Mediatech).

Cultures were maintained in T-75 flasks (Corning, Corning, NY) at 37 °C and a humidified environment of 5% CO_2 in air. The medium was changed every other day. Cells were routinely passaged at 80% confluency, which occurred between 6 and 7 days after seeding. A passage operation consisted of two washes with Dulbecco's phosphate buffered saline (DPBS) without Ca^{2+} and Mg^{2+} (Mediatech) and then the addition of 1 ml 0.5% trypsin/0.2% EDTA solution (Sigma, St. Louis, MO). Cells were then incubated with the trypsin/EDTA solution for 5 min, after which cells were detached from the flasks and could then be counted and reseeded.

Caco-2 cells were seeded at a density of 3.0×10^3 cells cm^{-2} and used between passages 60 and 80. HT29–MTX cells were seeded at a density of 2.0×10^4 cells cm^{-2} and used between passages 8 and 20.

2.3. Cytocompatibility

Cytocompatibility experiments were performed in 96-well plates (Corning) using both Caco-2 and HT29–MTX cells. Caco-2 cells were seeded at a density of 1.4×10^4 cells cm^{-2}, while HT29–MTX cells were seeded at a density of 2.8×10^4 cells cm^{-2}. Cells were fed every other day and cytotoxicity studies were conducted when cells reached 90% confluence (6–7 days).

P(MAA-*g*-EG) and P(MAA-*g*-EG) WGA were prepared as described. For this study the microparticles were sized between 90 and 150 μm. Growth medium was removed from each well and P(MAA-*g*-EG) and P(MAA-*g*-EG) WGA functionalized microparticles were added to the wells at concentrations ranging between 0.5 and 2.5 mg ml^{-1} in Hank's balanced salt solution (HBSS) (Mediatech). Prior to addition, the pH of each suspension was adjusted to 7.4 with 0.1 N NaOH. Microparticles were then incubated with the cells for 2 h at 37 °C and 5% CO_2. The microparticle suspension was removed from each well and the wells were rinsed three times with HBSS.

To determine cell viability, a cellular metabolic assay was used to measure NADPH production (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI). Results were compared with control wells that were not incubated with microparticles, but only with HBSS and the CellTiter 96® reagent.

2.4. Caco-2 Transwell® culture

All transport and transepithelial electrical resistance (TEER) experiments were conducted using a Costar Transwell® plate (Corning) with a polycarbonate membrane (0.4 μm pore size) and a cell growth area of either 4.7 cm^2 (6-well) or 1 cm^2 (12-well). Cells were seeded at a density of 6×10^4 (6-well) or 1×10^5 cells cm^{-2} (12-well) after a passaging procedure and cultured for 21–24 days. Medium was changed every other day and TEER values were measured with an EVOM volt-ohm meter and a chopstick electrode (World Precision Instruments, Sarasota, FL) to monitor development of tight junctions. It has been well documented that Caco-2 cells form an absorptive polarized monolayer, develop an apical brush border and secrete enzymes after culture for 21 days [2,5].

2.5. Caco-2/HT29–MTX Transwell® culture

Caco-2 and HT29–MTX cells were maintained separately in T-75 flasks as previously described. After subculturing, the cells were counted and mixed together in a 1:1 ratio before seeding onto the Transwell® plate at a density of 6×10^4 (6-well) or 1×10^5 cells cm^{-2} (12-well). Previous research demonstrated that a 1:1 seeding ratio produced TEER values closest to those reported in vivo for human intestinal epithelia [6]. As with the Caco-2 cells, medium was changed every other day in the co-culture and TEER was used to monitor the development of tight junctions.

2.6. TEER evaluation

TEER was used to evaluate the development of tight junctions in the Transwell® cultures. Measurements were taken every other day 2 h after changing the medium. In order to determine the resistance across the cellular monolayer ($R_{\text{true tissue}}$) it was important to subtract resistance due to the membrane and the medium within the wells. A blank resistance measurement was taken in the presence of medium without cells (R_{blank}) and then subtracted from the experimental TEER value.

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