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Targeted delivery of the hydroxylase inhibitor DMOG provides enhanced efficacy with reduced systemic exposure in a murine model of colitis

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ABSTRACT

Targeting hypoxia-sensitive pathways has recently been proposed as a new therapeutic approach to the treatment of intestinal inflammation. HIF-hydroxylases are enzymes which confer hypoxic-sensitivity upon the hypoxia-inducible factor (HIF), a major regulator of the adaptive response to hypoxia. Previous studies have shown that systemic (intraperitoneal) administration of hydroxylase inhibitors such as dimethyloxalylglycine (DMOG) is profoundly protective in multiple models of colitis, however the therapeutic potential of this approach is limited due to potential side-effects associated with systemic drug exposure and the fact that orally delivered DMOG is ineffective (likely due to drug inactivation by gastric acid). In order to overcome these issues, we formulated DMOG in a liquid emulsion drug delivery system which, when coated with specific polymer coatings, permits oral delivery of a reduced dose which is released locally throughout the colon. This colon-targeted DMOG formulation demonstrated increased relative colonic bioactivity with reduced systemic exposure and provided a similar degree of protection to systemic (intraperitoneal) administration at a 40-fold lower dose in DSS-induced colitis. In summary, targeted delivery of DMOG to the colon provides local protection resulting in enhanced efficacy with reduced systemic exposure in the treatment of colitis. This novel approach to targeting hydroxylase inhibitors to specific diseased regions of the GI tract may improve it's potential as a new therapeutic in inflammatory bowel diseases such as ulcerative colitis.

1. Introduction

Molecular oxygen (O_2) is the primary electron acceptor of the mitochondrial electron transport chain and as such, a sufficient oxygen supply is essential to maintain metabolic homeostasis and cell survival. Over the course of evolution, metazoans have developed a highly conserved and effective ability to respond to the metabolic threat of reduced oxygen (hypoxia) with the induction of an adaptive transcriptional response which promotes tissue oxygenation. The hypoxia-inducible factor (HIF) is a key regulator of this response which controls the expression of an array of genes that promote survival through adaptation to hypoxia. HIF-dependent genes encode proteins which promote hypoxia-adaptive processes such as angiogenesis (e.g. vascular endothelial growth factor), erythropoiesis (e.g. erythropoietin) and glycolytic metabolism (e.g. pyruvate dehydrogenase kinase).

2-oxoglutarate-dependent hydroxylases are a family of dioxygenases, a subset of which play an important role in cellular oxygen-sensing through the conferral of oxygen-dependence upon hypoxia-sensitive transcriptional regulators including the HIF and Nuclear factor kB (NF-κB) [1,2]. Pharmacologic hydroxylase inhibitors including the 2-oxoglutarate mimetic DMOG have been demonstrated to be protective in multiple models of intestinal inflammation including chemical-, toxin-, radiation- and ischemia/reperfusion-induced intestinal injury [3–10]. The mechanism(s) of anti-inflammatory action of hydroxylase inhibitors in the intestine are multifactorial and may be HIF-dependent or HIF-independent [11]. Firstly, it appears that hydroxylase inhibition, through the activation of a panel of HIF-dependent barrier protective genes including intestinal trefoil factor, Mucin-3, p-glycoprotein, ecto-5-nucleotidase and the adenosine A2B receptor leads to enhanced epithelial barrier function [12,13]. Secondly, hydroxylase inhibition in intestinal epithelial cells leads to a reduction in epithelial cell death through apoptosis which also contributes to enhanced intestinal barrier function [9,14]. Thirdly, in a HIF-independent manner, hydroxylase inhibitors decrease interleukin-1β-induced pro-inflammatory signaling and subsequent gene expression [15]. Fourthly, hydroxylase inhibition can impact upon inflammatory processes through the HIF-independent regulation of neutrophil survival [16]. Therefore, hydroxylase inhibitors represent a promising new therapeutic approach to the treatment of inflammatory

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diseases of the gastrointestinal tract through both HIF-dependent and HIF-independent mechanisms [17].

Most of the experimental models which have demonstrated protective effects of hydroxylase inhibitors in intestinal inflammation to date have utilized systemic administration of the drugs [17]. However, systemic exposure to hydroxylase inhibitors results in unwanted side effects such as erythropoiesis and angiogenesis. Furthermore, oral administration of DMOG is not effective against colitis. This lack of efficacy is believed to be due mainly to susceptibility of the drug to acidic and enzymatic degradation in the stomach and small intestine. To overcome these issues, we hypothesized that targeted delivery of low dose DMOG to the colon would be of benefit in treating colitis as it would provide local colonic tissue protection without the risks associated with systemic exposure. The formulation technology used in this study was developed to permit colon-targeting and thereby enables DMOG release throughout the colon where it is then available for to provide a topical therapeutic effect. To test this hypothesis, we utilized this colon-targeted, minisphere-based technology which has been developed to target cargo drug release selectively throughout the colon with minimal release in the small intestine. This system is directed towards maximizing colonic delivery of the drug while minimizing systemic exposure. We compared the effectiveness of an orally administered, colon-targeted formulation of DMOG with systemic (intraperitoneal) administration with respect to markers of disease progression in the DSS model of murine colitis. We found that colon-targeted DMOG demonstrated enhanced efficacy in the treatment of colitis at a lower dose with significantly reduced systemic activity. In summary, we demonstrate that colon targeted, non-systemic delivery of a hydroxylase inhibitor provides a novel efficacious and safe potential alternative therapeutic approach to treating ulcerative colitis.

2. Materials & methods

2.1. Preparation of minispheres

Minispheres were prepared according to the protocol detailed in the US patent register (patent # WO 2008/122967) and outlined in Table 1. The source and chemical grades of the components used for formulation are outlined in Supplementary Table 3. Briefly, DMOG (Caymen Chemicals, >98% purity), Sodium Dodecyl Sulfate, D-Sorbitol and Type A porcine gelatin were added to water under constant agitation with a standard magnetic stirrer at 100–250 rpm. This is referred to as the aqueous phase. Transcutol HP, Cremophor EL and Miglyol 810 N were stirred at room temperature with a standard magnetic stirrer at 100–250 rpm until a clear solution was obtained. This is referred as the oil phase. The oil phase was mixed with the aqueous phase in a 1:10 ratio to form an emulsion that was stirred using a standard magnetic stirrer at 100–250 rpm to achieve complete homogeneity. The homogenous emulsion was then ejected through a single orifice (approximately 1 mm diameter) to form droplets which solidify in cooling oil medium (Miglyol 810 N) to form beads which were recovered by passing through a 500 μm sieve and then dried overnight at room temperature. These minispheres are referred to throughout the manuscript as the "uncoated DMOG minispheres". Uncoated DMOG minispheres were weighed and coated with a combination of ethylcellulose:pectin (E:P) (98:2% w/w) using a Würster coater (Freund-Vector MFL.01 Micro Fluid Bed Dryer; Supplementary Fig. 1). The degree of E:P coating thickness was calculated as a function of % dry weight gain achieved. The weight gain achieved was 6.5%. These minispheres are referred to throughout the manuscript as "coated DMOG minispheres". The preparation efficiency using this approach is approximately 85% (input/output). Using this formulation approach, 90% of the minispheres harvested are within the 1.0–1.4 mm diameter range while 10% are between 1.4 and 2.0 mm in diameter. X-ray tomography and microscopic images of sample beads are shown in Supplementary Fig. 2.

Table 1
This table provides the details of each ingredient of the aqueous and oil phases used for the formulation of DMOG-containing minispheres.

Oil phase components	IUPAC name	Function
Transcutol HP	Diethylene glycol monoethyl ether	Bioavailability enhancer
Cremophor EL/Kolliphor EL	Polyoxyl 35 castor oil	Surfactant
Miglyol 810 N	Medium chain triglyceride	Oil
Aqueous phase components	**IUPAC name**	**Function**
Type A porcine gelatin 275-07	Gelatin	Matrix-forming polymer
Purified water	Water	Solvent
Sorbitol	Sorbitol	Plasticizer
SDS	Sodium dodecyl sulfate	Surfactant
DMOG	Dimethyloxalyl glycine	API

After the minispheres are orally administered, the ethylcellulose–pectin coating layer starts to wet and hydrate which initiates the creation of channels in the polymer coating; the thickness of the coat correlates with the time when the cores starts to dissolve and release the soluble and intact DMOG into the gastrointestinal lumen. Due to the coating thickness of these beads, the release is delayed until the beads reach the colon. Once in the large intestine the pectin, which is included as a pore-former in the ethylcellulose is degraded by the bacteria that are present in the colon. This degradation creates pores in the coating layer through which the drug is released, further enhancing the colon-targeted release of DMOG. The inclusion of the oil phase excipients further modulate the rate of dissolution of the core and support the interaction between the drug and the colon tissue.

2.2. Dissolution of minispheres

Minispheres were added to 1 ml Dulbecco's modified Eagle cell culture medium in 1.5 ml Eppendorf tubes. The tubes were placed on a rotating platform for 12 h to allow complete dissolution and DMOG release. The resulting mixture was centrifuged at 10,000 rpm for 5 min. The supernatant was collected and filtered through a 0.22 μm filter for sterile filtration (Millex-GP filter unit; Millipore).

2.3. Luciferase assay

Hela cells were transfected with a luciferase reporter construct encoding the firefly luciferase gene under the control of a concatemer of hypoxia response elements (HRE). Cells were cultured for 24 h before treatment with filtered supernatant containing the dissolved spheres for up to 6 h after which cell lysates were harvested at room temperature following washing using 200 ul of 1× luciferase lysis buffer (Promega, UK). Lysates were transferred to Eppendorf tubes and centrifuged at room temperature at 14,000 rpm for 6 min. 20 ul of lysate was added to 100 ul luciferase substrate solution (Promega, UK) and mixed vigorously using a pipette for 5 s. The 5 ml test-tube was immediately placed in a desktop luminometer (Berthold Technologies Junior LB 9509) and emitted light units were read over a 20 s period. The cumulative light units were displayed. The read-out is relative light units or RLU.

2.4. Oral administration of colon targeted spheres

The spheres were administered orally using a modified oral gavage needle (Supplementary Fig. S3). The sharp tip of a 16.5 gauge needle was cut and the edges were smoothened by a metal file. A 50 mm piece of clear, flexible PVC tube with the internal diameter 1.5 mm was fixed on the needle and secured using glue. The minispheres were loaded in the PVC tube. The needle was then fixed on to a 1 ml syringe containing 50 μl of PBS and 800 μl air. The needle and syringe assembly is positioned at the esopharangeal opening and the sphere was released