P-065	**Chitosan/alginate pellets with rutin developed for inflammatory bowel disease treatment**

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INTRODUCTION AND OBJECTIVES

Inflammatory bowel disease (IBD) is a refractory, chronic, recurrent and non-specific inflammatory disorder of intestinal tract. Rutin and other flavonoids exhibit anti-oxidant and anti-inflammatory effects; therefore they are the subject of interest as potential drugs for the treatment of several diseases including IBD (Rabiskova 2009). In our experiment, pellets based on chitosan/alginate for rutin colon delivery were prepared, evaluated *in vitro* and *in vivo* in experimental colitis in rats.

MATERIALS AND METHODS

Materials

Rutin (Sigma-Aldrich, Germany) as an active ingredient, chitosan (deacetylation degree of 91 %; JBiChem, China) and microcrystalline cellulose Avicel® PH 101 (FMC, Ireland) as fillers, and sodium alginate (Sigma-Aldrich, Germany) as the main coating excipient were used to prepare coated pellets. Acetate or phosphate buffers (pH 3.0, 6.8, 7.5, 4.0, 6.0, Ph. Eur. 6) and β-glucosidase (3811U/mg; MP Biomedicals, USA) were used for dissolution studies. Trinitrobenzensulfonic acid, hexadecyltrimethylammonium bromide (Fluka Biochemika, Germany) and o-dianisidine dihydrochloride (Sigma-Aldrich, Germany) were used in *in vivo* experiments.

Pellet preparation

Pellets containing 30% of rutin, 45% of chitosan and 25% of microcrystalline cellulose were prepared using extrusion/ spheronization method (Pharmex 35T, Wyss & Probst, Germany), subsequently coated with alginate/chitosan (95:5) solution in Wurster type fluid bed unit (M-100, Medipo, Czech Republic) up to 18 % of total pellets mass, and dried in ventilated oven (Horo, Germany) at 40 °C for 3 hours.

Pellet characterization

Pellet characteristics (mean diameter, sphericity, hardness, friability, Hausner ratio) were determined (Scala-Bertola 2009). Rutin content was measured spectrophotometrically at a wavelength of 360 nm (phosphate buffer pH 6.8; Lambda 25, Perkin Elmer Instruments, USA). Drug release was evaluated (1000 mL, 100 rpm, 37 ° C, 360 nm; Sotax AT 7 Smart on-line, Donau Lab, Switzerland) using basket dissolution method with changing pH values simulating gastrointestinal tract conditions. β-glucosidase was added to the phosphate buffer of pH 6.0 to decompose chitosan *in vitro* (Dvorackova 2011; Table 1).

Table 1: Simulating GIT conditions

Part of GIT	pH	Medium	Time
Stomach	3.0	Phosphate buffer	2 h
Small intesine	6.8	+ 4.2 g Na_3PO_4. 12 H_2O	3 h
Terminal ileum	7.5	+ 6.0 g Na_3PO_4. 12 H_2O	0.5 h
Colon	4.0/ 6.0	Acetate buffer/ phosphate buffer + β-glucosidase	16.5 h

Animal studies

Animal experiments (*Guide for the Care and Use of Laboratory Animals,* Institute of Laboratory Animal Resources, National Research Council and National Academy of Sciences, USA) with male Wistar rats (175-199 g; Société Janvier, Le Genest, France) in 5 groups (n=4) according to colitis and drug administration (healthy control group; colitis control group - non treated; rutin solution receiving group per rectum; rutin solution receiving group per os and rutin pellets receiving group per os) were provided after a week of acclimatization. Colitis was induced (Morris 1989) and developed for 2 days. Treated groups received rutin (10 mg/kg) once daily for 5 continuous days. Colitis controls received physiological saline orally instead. One day after the treatment, the animals were sacrificed and colons were resected. Colon/body weight ratio (Lamprecht 2005) and myeloperoxidase activity were determined (Krawisz 1984).

RESULTS AND DISCUSSION

Pellet evaluation in vitro

Pellets of very good characteristics: pellet mean diameter 0.77 mm, sphericity 0.83, hardness 9.35 N, friability 0.21 % and Hausner ratio 1.08, were obtained. Rutin content was 29 %, i.e. 97 % of its theoretical value. Coated pellets showed low rutin dissolution (14 %) under upper gastrointestinal tract conditions, while its release was fast (close to 90 %) under the conditions mimicking the colon; that is a good presumption of intended rutin release (Fig. 1).

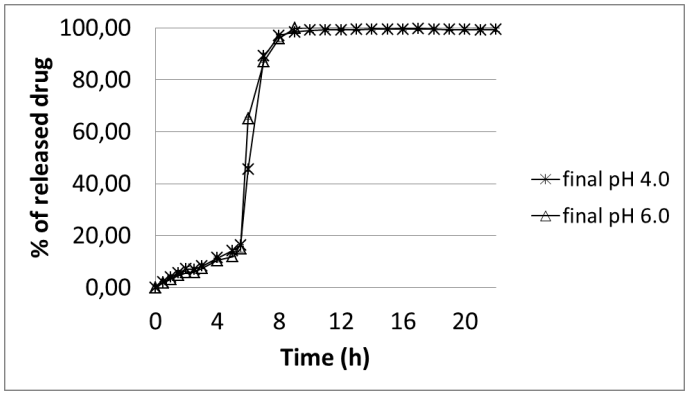

Figure 1: Rutin dissolution profiles

In vivo studies

In vivo experiment, rutin was able to promote colonic healing at the dose of 10 mg/kg, i.e. colon/body weight ratio decreased (Fig. 2) and myeloperoxidase activity was significantly suppressed (Fig. 3). The best colon/body weight ratio results (Fig. 2) were obtained in group receiving pellets (0.008) or rutin solution rectally (0.009). In healthy control group, colon/body weight ratio was also calculated and reached the value of 0.004. Concerning myeloperoxidase activity (Fig. 3), coated pellets showed excellent therapeutic efficiency and the value of 250 U/g tissue, i.e. myeloperoxidase activity reduction almost to the value of the control group.

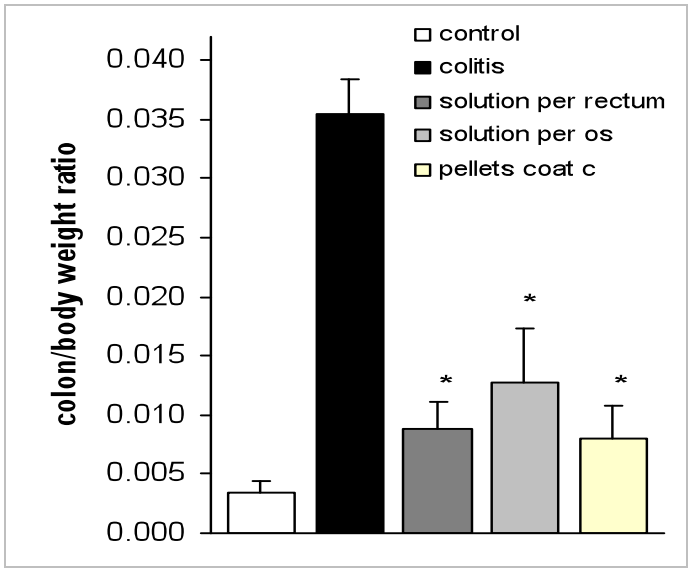

Figure 2: Colon/body weight ratio

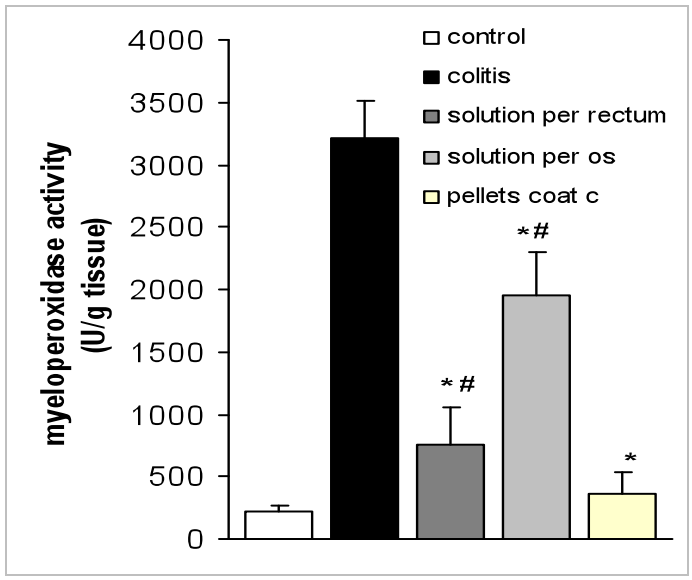

Figure 3: Myeloperoxidase activity

CONCLUSIONS

The combination of natural rutin, mucoadhesive chitosan degraded in the colon together with alginate coating, and pellets as multiparticulate dosage form having numerous advantages in IBD treatment could form a promising preparation free of side effects for lifelong therapy of this severe illness.

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