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The small intestinal mucosa acts as a rutin reservoir to extend flavonoid anti-inflammatory activity in experimental ileitis and colitis

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ABSTRACT

Flavonoids are considered versatile components in many functional foods with multiple health benefits. One of the most abundant flavonoids, rutin, is effective in experimental colitis, which is attributed to colonic intraluminal release of its flavonol quercetin. Surprisingly, however, quercetin is ineffective. We aimed to explore whether rutin/quercetin protect against trinitrobenzenesulphonic acid (TNBS) experimental ileitis. TNBS colitis was also studied for reference. Rutin was active in ileitis and in colitis, while quercetin showed only marginal effects. In order to explain this discrepancy we measured flavonoid mucosal levels and found that anti-inflammatory activity correlated with the presence of rutin in the ileal mucosa rather than with colonic mucosal levels of rutin or quercetin. In fact, rutin was protective against colitis even when administered intraperitoneally. Our results indicate that rutin is taken up by the ileal mucosa and slowly released to the lumen, resulting in extended exposure of the mucosa to quercetin.

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Abbreviations: AP, alkaline phosphatase; IBD, inflammatory bowel disease; MLNC, mesenteric lymph node cells; MPO, myeloperoxidase; TNBS, trinitrobenzenesulphonic acid; TNFα, tumor necrosis factor alpha; Treg, regulatory T cells

Chemical compounds: Rutin (PubChem CID: 5280805); Quercetin (PubChem CID: 5280343); Trinitrobenzenesulphonic acid (PubChem CID: 11045); Fisetin (PubChem CID: 5281614).

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1. Introduction

Flavonoids are polyphenolic compounds of natural origin that are consumed as part of the normal human diet. As much as 200–500 mg may be ingested daily in Western countries (McCullough et al., 2012; Russo, Spagnuolo, Tedesco, Bilotto, & Russo, 2012). These compounds are considered as functional food bioactives as many different biological properties have been ascribed to them, including immunomodulatory/anti-inflammatory activity (González et al., 2011). Importantly, a number of flavonoids, including morin, quercitrin, rutin, genistein or myricetin, have been shown to exert intestinal anti-inflammatory effects in preclinical models of inflammation (González et al., 2011; Shigeshiro, Tanabe, & Suzuki, 2013). Quercetin is a major flavonoid species in vegetables included in the human diet, although it is mostly present in its glycosylated forms, i.e. as glycosides such as quercitrin and rutin. Rutin is a quercetin rhamnoside (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4H-chromen-4-one) and its anti-inflammatory properties have been documented using *in vivo* experimental models of inflammatory bowel disease (IBD), asthma and rheumatoid arthritis (González et al., 2011). Among the disorders that involve intestinal inflammation, IBD, comprising essentially ulcerative colitis and Crohn's disease, is a chronic inflammatory condition of the gut with a prevalence of approximately 0.1% in developed countries (Kaser, Zeissig, & Blumberg, 2010). IBD has a high impact on patients, because it is difficult to manage, often requires surgery, and currently has no cure, with the only exception of total colectomy in ulcerative colitis. In addition, there are important side effects associated with the pharmacotherapy of IBD. Therefore, there is a substantial need for new strategies in this condition. Because of their anti-inflammatory and antioxidant properties and their low toxicity, the use of polyphenols in general, and of rutin in particular, may be valuable alternatives for the management of intestinal inflammation as functional foods (González et al., 2011; Shigeshiro et al., 2013).

An important characteristic regarding the use of quercetin is that its oral ingestion is essentially inactive on experimental models of colitis (Comalada et al., 2005; Kwon, Murakami, Tanaka, & Ohigashi, 2005). Instead, the glycoside derivatives of quercetin, rutin (Galvez et al., 1997) and quercitrin (Camuesco et al., 2004; Comalada et al., 2005; Sanchez de Medina, Galvez, Romero, & Zarzuelo, 1996) exert significant effects in *in vivo* models. The commonly accepted hypothesis is that these compounds are hydrolyzed by bacterial enzymes in the colon to yield quercetin (Comalada et al., 2005; Kim et al., 2005). It has been proposed that quercetin glycosylation may prevent absorption of the flavonoid in the small intestine, allowing it to reach the colon. Thus quercetin would be expected to exert protective effects in small intestinal inflammation. We aimed to verify the effect of quercetin vs. rutin in trinitrobenzenesulphonic acid (TNBS) ileitis. Our data show for the first time that rutin exerts significant protection in small intestinal inflammation, and, surprisingly, it was more effective than quercetin, as shown also in TNBS colitis. Since elucidation of the mechanism of action of candidate functional foods is important, in the present study we explored the reason for this discrepancy by measuring flavonoid mucosal levels and additionally by testing the effect of intraperitoneally administered rutin on TNBS colitis. This is also of interest as flavonoids have been proposed to complement parenteral nutrition in clinical gastroenterology (Hoensch & Oertel, 2011). Our results indicate that rutin is taken up by the ileal mucosa and slowly released to the lumen, resulting in extended exposure of the mucosa to quercetin.

2. Materials and methods

2.1. Materials and reagents

Except when otherwise indicated, all reagents were obtained from Sigma (Madrid, Spain). The corticoid budesonide was purchased from Molekula Ltd. (Gillingham, UK).

2.2. Animals

Female Wistar rats (175–225 g) supplied by Harlan (Barcelona, Spain) were housed in makrolon cages and maintained in a thermostatized room with a 12 h light–dark cycle. The animals had free access to tap water and standard chow diet (Panlab A04, Panlab, Barcelona, Spain). This study complied with national and international regulations and was approved by the Ethical Committee of the University of Granada.

2.3. Induction of trinitrobenzenesulphonic acid colitis and ileitis and experimental design

Colitis was induced as described previously (Daddaoua et al., 2005). Briefly, rats were fasted overnight and anesthetized with 1.7% isoflurane (200 mL/min airflow, 1 mL/h liquid flow). Under these conditions, rats were given 10 mg of trinitrobenzenesulphonic acid (TNBS) dissolved in 0.25 mL of 50% ethanol (v/v) by means of a Teflon cannula inserted 8 cm through the anus. Two separate colitis experiments were carried out initially, in which rats were randomly assigned to one of 4 different groups, namely control (n = 4), TNBS (n = 6–8), intragastric budesonide (2 mg/kg, n = 8), and either intragastric rutin (n = 6) or intraperitoneal quercetin (n = 6). Rutin and quercetin were administered in equimolar doses (20 and 11.1 mg/kg, respectively, equivalent to 32.8 μmol/kg body weight). All groups received the TNBS challenge, except the control, which was administered a saline enema. All treatments started 2 days before colitis induction and were maintained until animals were sacrificed (isoflurane overdose), after 7 days. Food and water intake and body weight were determined daily.

Additional experiments were carried out with an intraperitoneal rutin (20 mg/kg) and a separate intragastric budesonide treatment (2 mg/kg), following the same protocol. The dose of budesonide, a corticoid used as reference treatment, is based on the human dose, considering that the effect is local, and also on previous experiments from our group. The results shown are normalized data from two separate experiments presented as a single run (n = 8 for the control group and 7–14 for the rest of experimental groups).

Ileitis was induced by direct injection of 30 mg of TNBS in 50% ethanol in the ileum of isoflurane anesthetized rats. Control