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Antidiarrhoeic activity of quercitrin in mice and rats

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Abstract—Quercitrin, a flavonoid isolated from *Euphorbia hirta*, shows antidiarrhoeic activity at doses of 50 mg kg^{-1}, against castor oil- and PGE_2-induced diarrhoea in mice, but not when magnesium sulphate is used as a cathartic agent. It also delays rat small intestinal transit if this is accelerated with castor oil. However, the flavonoid does not modify the fluid transport across the colonic mucosa when it is administered intraluminally, either in normal conditions or when this transport is altered by PGE_2 or sodium picosulphate. However, quercetin, the aglycone of quercitrin, increases the colonic fluid absorption only in the presence of secretagogue compounds, such as PGE_2 and sodium picosulphate. It is concluded that the antidiarrhoeic activity of quercitrin is due to its aglycone, quercetin, which is released by the glycoside in the intestine.

Flavonoids are natural products which exhibit several pharmacological effects (Pathak et al 1991). In the gastrointestinal system, flavonoids lengthen the small intestine transit time (Meli et al 1990), inhibit the amplitude of phasic contractions and decrease the tone of guinea-pig isolated ileum (Abdalla & Abu Zarga 1987), and antagonize the contractions induced in isolated intestinal preparations by several agents, involving prostaglandin E_2, acetylcholine, barium chloride, and various antigens (Capasso et al 1991). Flavonoids inhibit several enzymes, including those involved in arachidonic acid metabolism (Landolfi et al 1984; Ferrandiz & Alcaraz 1991). Both mucosa and the muscle layers of the gut possess the ability to generate all major products of arachidonate metabolism via the cyclooxygenase pathway such as prostaglandins (Konturek et al 1985). Prostaglandins play an important role in diarrhoeal diseases. The administration of exogenous prostaglandins in man exerts potent stimulatory effects on intestinal secretion and propulsive motility, which are thought to cause diarrhoea (Rask-Madsen & Bukhave 1981). It has also been suggested that laxatives may act, at least in part, by stimulating prostaglandin synthesis (Bennet & Sanger 1982; Capasso et al 1986).

Quercitrin is a flavonoid, 3-rhamnosyl-quercetin, isolated from *Euphorbia hirta*. Quercitrin shows antidiarrhoeic activity at doses of 25 mg kg^{-1} against castor oil-induced diarrhoea in mice (Galvez et al 1993). The aim of the present paper was to study the possible mechanism involved in the antidiarrhoeal action exerted by quercitrin. For this purpose, quercitrin was tested against several models of experimentally-induced diarrhoea, using as cathartic agents, castor oil, prostaglandin E_2 (PGE_2) and magnesium sulphate. The activity of this flavonoid on small intestinal transit and on net fluid transfer across the intestinal mucosa, both in normal conditions and when these were altered by laxative agents, was also assayed.

Materials and methods

Experimentally-induced diarrhoea. Female Swiss Offi mice, 20–25 g, were used after 24 h food deprivation. Quercitrin, at doses of 50 mg kg^{-1}, was administered orally to groups of ten mice 60 min before the administration of the cathartic agents. The cathartic agents were castor oil (0·5 mL, p.o.), magnesium sulphate (2 g kg^{-1}, p.o.) and PGE_2 (1 mg kg^{-1}, i.p.). Following administration the animals were placed separately in polythene cages with filter paper, previously weighed, at the bottom, which was changed every 30 min. The following parameters were determined: the time elapsed between the administration of the cathartic agents and the excretion of the first diarrhoeic faeces (wet faeces that leave a halo on the filter paper); the total number of faeces as well as the number of diarrhoeic faeces excreted by the animals in 4 h; and the total weight of diarrhoeal stools in that period of time.

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Small intestine transit. The technique proposed by Leng-Peschlow (1986) was used in groups of eight female Wistar rats, weighing approximately 200 g each, which were fasted for 24 h. A suspension containing 10% active charcoal in 1·5% arabic gum was used as a marker. Test solution (1 mL) was administered orally 60 min after the oral administration of quercitrin or quercetin, at doses of 25 and 50 mg kg^{-1}. The rats were killed after 30 min, the small intestine rapidly and carefully removed, and the length traversed by the charcoal marker was calculated as a percentage of the total intestine length. The inhibitory action of quercitrin and quercetin on stimulated small intestinal transit were also studied by giving castor oil, 1·0 mL per rat, along with the active charcoal–arabic gum suspension.

Rat tied-off colon. The method proposed by Beubler & Juan (1979) was followed, and modified to the experimental needs. Groups of eight female Wistar rats, weighing approximately 200 g, were fasted 24 h before the experiments. The animals were anaesthetized with Nembutal (40 mg kg^{-1}, i.p.), and the entire colon rinsed carefully with 50 mL warm Tyrode solution; afterwards, air was injected to remove the fluid. Immediately after cleaning, the colon was filled with 2·5 mL Tyrode solution, and ligated at both ends. Quercitrin and quercetin were given intraluminally by adding various concentrations of the drugs to the Tyrode solution with which the colonic loop was filled. In another set of experiments, PGE_2 (0·12 mM) or sodium picosulphate (0·45 mM) was also added to the Tyrode solution in order to inhibit the fluid absorption in the colonic mucosa.

Other experiments were made without rinsing the colon lumen with Tyrode solution, according to the method proposed by Beubler & Badhri (1990).

Net fluid transfer rates were determined gravimetrically 30 min after instillation of Tyrode solution for all the experiments. Net fluid transport was expressed as mL/30 min (g dried colon)$^{-1}$. Net absorption was indicated by a negative value and net secretion by a positive value.

Antioxidant properties. Inhibition of enzymatic lipid peroxidation in liver microsomes. Enzymatic lipid peroxidation was induced in liver microsomes (0·2 mg protein mL^{-1}) by an NADPH-Fe^{3+}-ADP system (400 μM NADPH, 50 μM Fe^{3+}, 4 mM ADP, 10 mM KH_2PO_4) at 37°C. Lipid peroxidation was assessed as thiobarbituric reactive substances (TBARS), as described by Esterbauer & Cheeseman (1990), after 15 min. Liver microsomes were isolated according to Albro et al (1987) and the proteins were measured by the method of Bradford (1976). Quercitrin and quercetin were dissolved in a water/DMSO (7:3) mixture and added to samples in a concentration range of 1–100 μM to determine the IC50 values. Control samples also contained an equal volume of the water/DMSO mixture.

Table 1. Antidiarrhoeic activity of quercitrin (50 mg kg^{-1}) against experimentally-induced diarrhoea in mice.

Cathartic agent	Time (min)	Total number of faeces	Number of wet faeces	Total weight of wet faeces (mg)
Magnesium sulphate (2 g kg^{-1}, p.o.)				
Control	144±9	24·0±2·7	17·2±2·2	278±28
Quercitrin	114±30	24·2±1·7	17·4±1·4	323±57
Prostaglandin E_2 (1 mg kg^{-1}, i.p.)				
Control	7±1	14·4±1·4	12·2±1·2	867±39
Quercitrin	12±2*	11·9±1·9	9·2±1·1	733±43*
Castor oil (0·5 mL, p.o.)				
Control	59±8	22·7±1·3	18·2±1·8	46±26
Quercitrin	101±16**	18·0±0·9**	11·2±2·2**	203±39*

Each value represents the mean ± s.e.m. obtained from 10 animals. *$P<0.05$; **$P<0.01$ compared with the corresponding control.

Statistics. The results were expressed as arithmetic means with standard error of the mean (s.e.m.). Significance of the differences in comparison with the control groups was determined with Student's *t*-test. Values of IC50 for the inhibition of lipid peroxidation were calculated from the regression equations where x = log concentration of a flavonoid; y = per cent of its inhibitory effect.

Chemicals. Chemicals and reagents used were: quecitrin and quercetin (both from Extrasynthese, Bordeaux, France); castor oil (Acofarma, Madrid, Spain); magnesium sulphate (Merck, Darmstadt, Germany); sodium picosulphate (Boehringer Ingelheim, Barcelona, Spain); PGE_2, Nembutal, trichloroacetic acid, thiobarbituric acid, $FeCl_3$ (all from Sigma, Barcelona, Spain); NADPH and ADP (both from Boehringer Manheim, Madrid, Spain). All other reagents were of analytical grade.

Results

Antidiarrhoeic activity. Quercitrin, at doses of 50 mg kg^{-1}, showed antidiarrhoeic activity (Table 1) in castor oil- and in PGE_2-induced diarrhoea. When quercitrin was tested against castor oil, it significantly decreased both the faecal output (total number of faeces and number of diarrhoeic faeces, $P<0.01$) and the weight ($P<0.05$) of diarrhoeic faeces excreted for 4 h. However, when quercitrin was assayed using PGE_2 as cathartic agent, only the weight of diarrhoeic stools was significantly reduced ($P<0.05$). The flavonoid showed no effect if the diarrhoea was provoked by magnesium sulphate.

Small intestine transit. At doses of 25 and 50 mg kg^{-1}, quercitrin did not modify the normal intestinal transit. The percentage of the total length of the small intestine traversed by the charcoal meal was 59·2±3·9 and 57·8±2·8% at doses of 25 and 50 mg kg^{-1} (57·9±4·1% for the control group). However, quercitrin produced a significant decrease in the distance travelled by the marker suspension when the small intestinal transit was accelerated with castor oil, from 71·8±1·9% in the control group to 52·3±3·5 ($P<0.01$) and to 53·1±3·2% ($P<0.01$) at doses of 25 and 50 mg kg^{-1}, respectively.

Oral administration of quercetin, at doses of 25 and 50 mg kg^{-1}, did not affect either the normal transit (58·9±1·1 and 62·0±3·8%, respectively) or the stimulated transit with castor oil (75·5±3·8 and 75·8±2·9%, respectively).

Net fluid transfer in the rat tied-off colon. Quercitrin, when administered intraluminally, affected neither the fluid absorption in controls nor the altered transport produced by the addition of PGE_2 or sodium picosulphate to the Tyrode solution, at concentrations of 10, 100 and 1000 μM (Table 2).

Table 2. Effects of quercitrin and quercetin on net fluid transport in the rat tied-off colon, previously rinsed, in basal conditions and modified by PGE_2 (0·12 mM; $P<0.001$ compared with basal) and sodium picosulphate (0·45 mM; $P<0.001$ compared with basal).

	Basal	PGE_2	Sodium picosulphate
	(mL/30 min g^{-1})		
Control	−3·35 ±0·38	−1·74 ±0·26	−0·82 ±0·29
Quercitrin (μM)			
10	−2·43 ±0·40	−1·45 ±0·22	−0·95 ±0·54
100	−2·96 ±0·42	−1·63 ±0·35	−0·43 ±0·34
1000	−2·79 ±0·53	−1·57 ±0·29	−0·96 ±0·43
Quercetin (μM)			
10	−3·06 ±0·49	−2·09 ±0·41	−2·20 ±0·39*
100	−2·85 ±0·13	−3·59 ±0·44**	−3·93 ±0·46***

Values represent the mean ± s.e.m. of seven experiments. Net absorption is indicated by a negative value and net secretion by a positive value. *$P<0.05$, **$P<0.01$, ***$P<0.001$ compared with the corresponding control.

Quercetin, at concentrations of 10 and 100 μM, modified the net fluid transport across the colonic mucosa when this was altered by the presence of the laxative agents in the colonic lumen, without showing any effect in normal conditions (Table 2).

In those experiments in which the colon was not previously rinsed with Tyrode solution, quercitrin, at doses of 100 μM, did significantly increase the net fluid absorption when this was inhibited by sodium picosulphate (0·45 mM). In basal conditions the net fluid transport was −2·84±0·72 mL/30 min g^{-1}; when sodium picosulphate was added to the Tyrode solution, it produced a net secretion of fluid (+0·90±0·49 mL/30 min g^{-1}; $P<0.001$ compared with basal). However, if both quercitrin and sodium picosulphate were added to the Tyrode solution, the net secretion was transformed to net absorption (−2·21±0·38 mL/30 min g^{-1}), without showing significant differences compared with basal conditions.

Antioxidant properties. Both flavonoids prevented non-enzymatic lipid peroxidation in rat liver microsomes, quercetin: IC50 = 2·52 μM, quercitrin: IC50 = 13·6 μM.

Discussion

The activity shown by quercitrin on intestinal transit may be

summarized as follows: firstly, it delays small intestinal transit if it is accelerated with castor oil, but not in normal conditions; and secondly, it presents antidiarrhoeic activity against those models of experimentally-induced diarrhoea which are related with active modifications in the electrolyte and water transport across the intestinal mucosa. The results obtained would lead us to attribute the antidiarrhoeic activity showed by quercitrin to an increase in the electrolyte and fluid absorption by the intestinal mucosa, but only when the physiological processes involved in this transport are altered by agents like prostaglandins.

Prostaglandins contribute to the pathophysiological functions in the gastrointestinal tract (Bennet & Sanger 1982). Castor oil increases peristaltic activity and produces permeability changes in the intestinal mucous membranes to electrolyte and water, effects associated with prostaglandin release (Luderer et al 1980; Capasso et al 1986). Flavonoids present antioxidant properties (Mora et al 1990). This antioxidant activity is presumed to be responsible for the inhibitory effects exerted by flavonoids on arachidonic acid metabolism (Gryglewski et al 1987).

When quercitrin is assayed in the rat tied-off colon, the results obtained show that this flavonoid, at concentrations of 10, 100 and 1000 μM, does not modify the fluid transport across the colonic mucosa either in controls or when the transport is altered by the addition of PGE_2 or sodium picosulphate to the Tyrode solution with which the colonic loop is filled. The lack of activity in these experiments can be explained if, as with other glycosides (such as anthraquinones), the active aglycone has to be released by hydrolysis of the sugar. When quercetin, the aglycone of quercitrin, is tested in the rat tied-off colon in the same conditions used for quercitrin, it increases the colonic fluid absorption when this is inhibited by sodium picosulphate or PGE_2. On the other hand, when quercitrin is assayed in the same preparation without previously rinsing the colon lumen with Tyrode solution, the glycoside increases the absorption in the presence of sodium picosulphate. Goldhill et al (1989) and Beubler & Badhri (1990) point out that some drugs have to be reduced by intestinal contents to produce an effect, and this depends on rinsing of the colon lumen. These results can lead us to think that quercitrin must be metabolized by intestinal contents to release quercetin, and this aglycone is able to act on the intestinal processes involved in the fluid transport across the colonic mucosa, especially when these are altered by secretagogue compounds.

The inhibition in the prostaglandin synthesis exerted by this flavonoid (Gryglewski et al 1987) explain its antidiarrhoeic activity as it increases the absorption when this is inhibited by sodium picosulphate. However, it must also act by another mechanism not related to prostaglandin synthesis and release, as it is also active when PGE_2 is used to inhibit the fluid absorption across the colonic lumen, although higher doses of flavonoid are required to obtain this effect.

When quercitrin and quercetin are tested for their antioxidant properties by inhibition of enzymatic lipid peroxidation in rat liver microsomes, the results obtained confirm that quercetin is the active component of quercitrin, since the aglycone is 6·6 times more potent as an antioxidant agent than the glycoside.

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