Physiol. Res. 50: 501-506, 2001

Protective Effect of Quercetin on Ischemia/Reperfusion-induced Gastric Mucosal Injury in Rats

J. MOJŽIŠ, K. HVIŠČOVÁ, D. GERMANOVÁ, D. BUKOVIČOVÁ, L. MIROSSAY

Department of Pharmacology, Medical Faculty, Šafárik University, Košice, Slovak Republic

Received December 11, 2000
Accepted February 6, 2001

Summary

This study was designed to determine the gastroprotective properties of quercetin in ischemia/reperfusion-induced gastric mucosal injury and the involvement of endogenous prostaglandins in this process. Oral pretreatment of rats with quercetin (100 $mg.kg^{-1}$) 30 min before surgery significantly decreased the length of gastric mucosal lesions. However, lower doses of quercetin (25 and 50 $mg.kg^{-1}$) only slightly decreased the gastric mucosal injury. Intraperitoneal application of indomethacin (5 $mg.kg^{-1}$) had no effect in control (sham-operated) animals, but significantly worsened gastric injury in non-treated animals after ischemia/reperfusion. Furthermore, indomethacin only slightly reversed protective effect of quercetin. Non-treated animals showed a marked decrease in adherent mucus after ischemia/reperfusion. On the other hand, application of quercetin prevented this significant decrease even in animals pretreated with indomethacin. It can be concluded that antioxidant properties of quercetin and its mucus protective effect might be the main factors responsible for its protective effect against ischemia/reperfusion-induced gastric mucosal injury.

Key words
Quercetin • Gastric mucosa • Ischemia/Reperfusion • Indomethacin • Rats

Introduction

Flavonoids are a group of about 4000 naturally occurring polyphenolic compounds ubiquitously found in fruits and vegetables. They have a broad scale of biological effects and their antineoplastic (Matsuzaki *et al.* 1996), antimutagenic (Calomme *et al.* 1996), antiinflammatory (Noreen *et al*. 1998), antidiabetic (Perez *et al.* 1998), antihistaminic (Yamamura *et al.* 1998) and other effects (Mojžišová *et al*. 2000) have been described. Flavonoids may exert antioxidant effects as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers and metal ion chelators (Rice-Evans *et al.* 1995, Mojžišová *et al.* 1999). Certain flavonoids or compounds with flavonoid-like properties have been shown to possess antiulcer activity and were able to prevent gastric mucosal lesions produced by various ulcer-producing methods (Alacrón de la Lastra *et al.* 1992, Martin *et al.* 1993, Mirossay *et al.* 1999).

One of the most abundant natural flavonoids, present in a large number of fruits and vegetables, is quercetin (Kuo *et al.* 1998). It has been reported to prevent gastric mucosal ulceration in animal models including cold-restraint stress, pyloric occlusion (Martin

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

et al. 1993) or administration of absolute ethanol (Alacrón de la Lastra *et al*. 1994).

This study was designed to determine the effect of quercetin on ischemia/reperfusion-induced gastric mucosal injury. Mucosal lesions were evaluated macroscopically and the role of endogenous prostaglandins was also determined.

Methods

Male Wistar rats weighing 180-200g were used in this study. All animals were housed in cages with a mesh-floor to prevent the ingestion of hair and feces. They were fasted for 24 h before the experiment but allowed free access to water. On the day of experimentation, the animals were randomized into eight groups (10 rats per group). Group I – controls (sham operation); group II – animals were subjected to ischemia/reperfusion with no drug administration; groups III, IV and V – animals were subjected to ischemia/reperfusion with oral quercetin pretreatment 30 min before surgery in the dose 25, 50 or 100 $mg.kg^{-1}$, respectively.

To establish the role of endogenous prostaglandins in the protective effect of quercetin, experimental animals were pretreated with 5 $mg.kg^{-1}$ of indomethacin i.p., 60 min before ischemia/reperfusion (groups VI, VII, and VIII). Otherwise, the animals in these groups were treated in the same manner as in groups I, II, and V, respectively.

The rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 $mg.kg^{-1}$). The animals from group II, III, IV, V, VII and VIII were subjected to 30 min ischemia induced by occlusion of the coeliac artery followed by 30 min of reperfusion. The coeliac artery was clamped using an atraumatic microvascular clamp.

Adherent gastric mucus was determined by the method of Corne *et al.* (1974). Shortly, the stomach was removed, opened along the great curvature and rinsed in cold saline. The glandular part of the stomach was excised, weighed and immersed for 2 h in 10 ml of 0.1 % w/v Alcian blue (Sigma) in 0.16 $mol.l^{-1}$ sucrose solution. The excess dye was removed by two rinses in 0.25 $mol.l^{-1}$ of sucrose (15 min each). The mucus-bound dye was extracted by immersing the gastric tissue in 0.5 $mol.l^{-1}$ $MgCl_2$ solution, which was intermittently shaken for 1 min at 30 min intervals during a 2 h period. The blue extract was shaken with diethylether. The emulsion was then centrifuged at 3600 rpm for 10 min and the optical density of the aqueous phase was measured spectrophotometrically at 600 nm. The results are expressed as absorbance per gram of wet tissue (A/g of tissue).

At the end of the period of ischemia/reperfusion, the extent of gastric lesions was measured, and the length of lesions was expressed in mm.

The statistical significance of the difference between means was estimated by Student's t-test. The $p<0.05$ was selected as the limit of statistical significance.

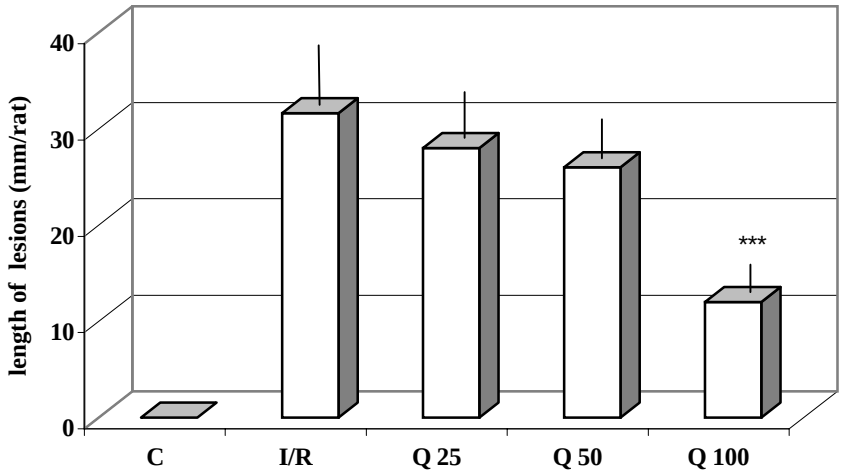

Fig. 1. *Effect of quercetin in the doses 25, 50 and 100 $mg.kg^{-1}$ (Q 25, Q 50 and Q 100) on ischemia/reperfusion-induced gastric mucosal injury. C – control (sham-operated animals), I/R – ischemia/reperfusion alone, ***p<0.001 I/R vs. Q 100*

Results

Our results have indicated that gastric injury was significantly increased after 30 min reperfusion following 30 min of ischemia by occlusion of the coeliac artery. The total length of gastric lesions reached a value of 31.6 mm/rat. Treatment with 100 mg.kg^{-1} of quercetin clearly diminished the extent and severity of mucosal injury (12 mm/rat; group V vs group II) ($p<0.001$). However, the protective effect of quercetin in both lower doses (25 and 50 mg.kg^{-1}, group III and IV) was only slight and non-significant. No macroscopic lesions were detected in sham-operated animals (group I) (Fig. 1).

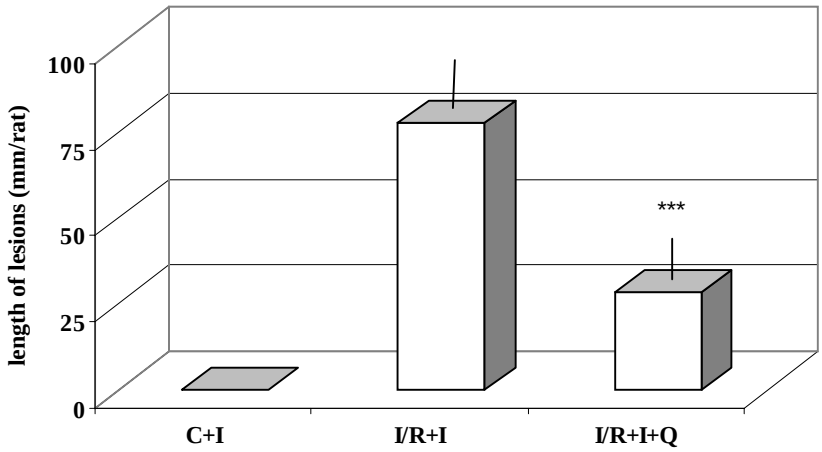

Fig. 2. *Effect of indomethacin (I) and quercetin in the dose 100 mg.kg^{-1} (Q) on ischemia/reperfusion-induced gastric mucosal injury. C+I – sham-operated animals after indomethacin (5 mg.kg^{-1}) pretreatment; I/R+I – ischemia/reperfusion in animals pretreated with indomethacin; I/R+I+Q – ischemia/reperfusion in animals pretreated with indomethacin and quercetin (100 mg.kg^{-1}); ***p<0.001*

The effect of intraperitoneal administration of indomethacin (5 mg.kg^{-1}) on gastric mucosal injury and the protective effect of 100 mg.kg^{-1} of quercetin is shown in Figure 2. Indomethacin significantly worsened the mucosal damage and the average length of mucosal lesions reached a value of 71.5 mm/rat. The administration of quercetin significantly reduced gastric mucosal injury in indomethacin-pretreated animals (average length of lesions was 28.5 mm/rat) (group VII vs. group VIII; $p<0.001$). No macroscopic lesions were found in sham-operated animals after indomethacin pretreatment (group VI).

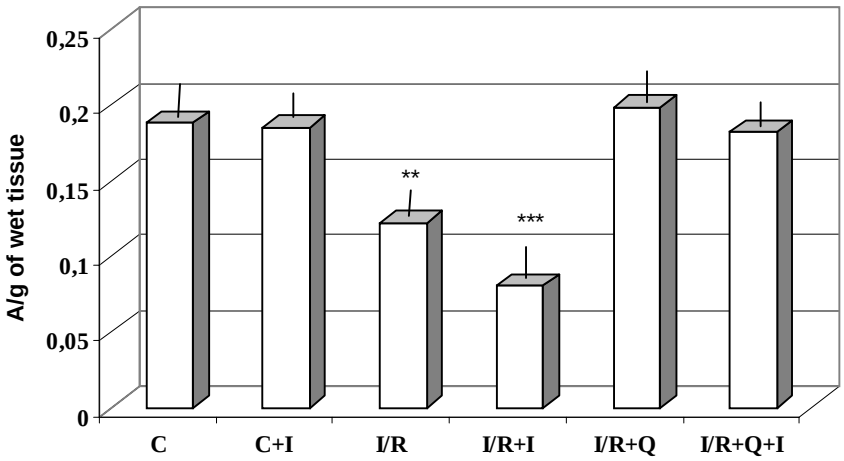

Fig. 3. *Effect of quercetin (Q) and indomethacin (I) on adherent mucus content. C – control (sham-operated animals), C+I control animals after indomethacin pretreatment, I/R – ischemia/reperfusion alone, I/R+I – ischemia/reperfusion after the indomethacin pretreatment, I/R+Q – ischemia/reperfusion after quercetin pretreatment, I/R+Q+I – ischemia/reperfusion after quercetin and indomethacin pretreatment, **p<0.01; ***p<0.001*

As shown in Figure 3 adherent mucus significantly decreased in both groups of animals after ischemia/reperfusion with no quercetin pretreatment (group II and group VII) in comparison with sham-operated animals (group I and group VI; $p<0.01$ and $p<0.001$, respectively). In quercetin-pretreated animals (group V and VIII) only a slight, non-significant decrease in mucus content was observed.

Discussion

Imbalance between mucosal defense and aggressive factors may result in acute gastric injury. It is known that gastric mucosal perfusion is an essential

factor in the ability of the mucosa to protect itself against injury (Cheung 1984). Later, it was found that reperfusion of ischemic tissue leads to tissue injury and it is believed that the main factors responsible for ischemia/reperfusion-induced injury are oxygen-free radicals (Zimmerman and Granger 1994) and polymorphonuclear leukocytes (Andrews *et al.* 1994). Hence, antioxidants or "anti-neutrophil" compounds are capable of eliminating their destructive effects (Mojžiš *et al.* 1996, Wada *et al*. 1996).

As has been mentioned above, quercetin possesses many biological effects. It is assumed that a number of them is due to its antioxidant activity. Quercetin scavenges oxygen radicals (Miller 1996), inhibits xanthine oxidase (Chang *et al.* 1993) and reduces lipid peroxidation (Chen *et al.* 1990). Furthermore, quercetin has been shown to down-regulate lymphocyte and natural killer-cell cytotoxicity and neutrophil function (Middleton and Kandaswami 1993).

In addition to its direct antioxidant effect, quercetin could protect the gastric mucosa by another mechanism. The gastric epithelium is covered by a continuous layer of secreted mucus and bicarbonate which have been widely implicated as important pre-epithelial protective factors against autodigestion of the gastric mucosa by acid and pepsin (Copeman *et al.* 1994). It has been reported that diminished gastric mucus renders the mucosa more susceptible to injury induced by various aggressive factors (Nosáľová *et al.* 1991, Leonard *et al.* 1994).

It has recently been suggested that gastric mucus may possess antioxidant properties (Grisham *et al.* 1987). Hiraishi and co-workers (1993) described a protective effect of mucus glycoproteins against oxygen-free radicals derived from the xanthine-xanthine oxidase system.

As was mentioned above, the gastric mucus plays an important role in the protection of the gastric mucosa. It is known that many sugars (e.g. manitol, glucose) are potent oxygen radical scavengers. On the basis of this fact, it was hypothesized that the gastric mucus would possess antioxidant properties because of the high glycoprotein content (Cross *et al.* 1984). The authors tested antioxidant effect of mucus glycoproteins and they found that 12 $mg.kg^{-1}$ solution of these proteins scavenged hydroxyl radical as effectively as 10-15 $mmol.l^{-1}$ solution of manitol or glucose. It was previously reported that gastric mucus contains glycoproteins at concentrations as high as 50 $mg.ml^{-1}$ (Allen 1981). Later, Hiraishi *et al*. (1993) confirmed the protective role of mucus glycoproteins against oxygen-free radicals in a cultured gastric epithelial cell system. Gong *et al.* (1990) reported that not only mucus glycoproteins possess the antiradical properties but also lipids bound to gastric mucin that protect it from oxygen radical attack. Recently, we have found that drugs able to stimulate mucus production also possess strong antiulcer activity and protect gastric mucosa against ischemia/ reperfusion-induced injury (Mojžiš *et al.* 2000). Our present results also support the suggested role of mucus in the protection of gastric mucosa. We found a significant decrease of adherent mucus content in animals after ischemia/reperfusion. On the other hand, pretreatment of animals with quercetin prevents mucus degradation under these experimental conditions and a significant protective effect of quercetin is also seen in these animals. Our results are in agreement with the results of Alacron de la Lastra and co-workers (1994) who found strong inverse relation between mucus content and gastric mucosal injury.

Our results and the above mentioned studies indicate that gastric mucus may be involved in the protection of gastric mucosa against oxygen radicals generated during ischemia/reperfusion. To assess a role of endogenous prostaglandins in gastroprotective effect of quercetin, we suppressed prostaglandin production with non-ulcerogenic dose of indomethacin (5 $mg.kg^{-1}$). Our results showed that despite of prostaglandin inhibition, the protective effect of quercetin was only slightly decreased. On the basis of these results, we suggest that endogenous prostaglandins probably play only a minor role in gastroprotective effect of quercetin in ischemia/reperfusion-induced gastric mucosal injury.

In conclusions, our results suggest that the antioxidant properties of quercetin and its mucus protective effect might be the main factors responsible for its strong protective effect on ischemia/reperfusion-induced gastric mucosa injury.

Acknowledgements

This work was partially supported by the Grant Agency of the Ministry of Education of the Slovak Republic and the Slovak Academy of Sciences No 1/6034/99.

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Reprint requests

Assoc. Prof. Ján Mojžiš, Ph.D., Department of Pharmacology, Medical Faculty, P.J. Šafarik University, Tr. SNP 1, 040 66 Košice, Slovak Republic, fax: ++ 421 95428524, e-mail: mojzis@central.medic.upjs.sk