European Journal of Pharmacology 698 (2013) 379–387

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Comparative study of the quercetin, ascorbic acid, glutathione and superoxide dismutase for nitric oxide protecting effects in mouse gastric fundus

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ARTICLE INFO

Article history:
Received 21 June 2011
Received in revised form
25 September 2012
Accepted 8 October 2012
Available online 17 October 2012

Keywords:
Antioxidants
Quercetin
Gastric fundus
Nitric oxide protecting effect
Superoxide generators
Tissue total antioxidant capacity

ABSTRACT

The aim of this work was to compare the preventing capacity of quercetin with Cu/Zn superoxide dismutase (Cu/Zn SOD), ascorbic acid and glutathione on nitric oxide (NO)-induced relaxation in mouse gastric fundus. Furthermore, the effects of the quercetin on the tissue level of total oxidant and antioxidant was investigated. Nitrergic stimulation (4 Hz, 25 V, 0.1 ms, 10 s-train) and exogenous NO (10 μM) induced relaxation. Pyrogallol (10 μM), hydroquinone (100 μM) and LY83583 (6-Anilinoquinolin-5,8-quinone, 5 μM) inhibited nitrergic relaxations. The inhibition observed with pyrogallol, hydroquinone and LY83583 was prevented by quercetin (0.1 μM). Also, ascorbic acid (500 μM), glutathione (100 μM) and Cu/Zn SOD (100 U/ml) prevented the inhibitory effect of superoxide anion generators on the relaxation to nitrergic stimulation and NO. Diethyldithiocarbamic acid (DETCA; 8 mM) inhibited nitrergic relaxations. DETCA-induced inhibition on nitrergic stimulation and NO-induced relaxation was prevented by quercetin, ascorbic acid, glutathione or Cu/Zn SOD. DETCA plus pyrogallol, hydroquinone or LY83583 strengthened the inhibition on the relaxations. Also, pre-treatment with quercetin, ascorbic acid and glutathione prevented the inhibitory effect of DETCA plus LY-83583 on the relaxation to nitrergic stimulation and NO but Cu/Zn SOD did not prevent this inhibition. Also, quercetin increased tissue total antioxidant capacity and decreased tissue oxidant level and oxidative stress index in DETCA-treatment group. These results indicate that quercetin has antioxidant effect and protects NO from endogenous superoxide anion-driven inactivation and enhances its biological activity, suggesting that quercetin may scavenge superoxide anion in a Cu/Zn SOD, glutathione or ascorbic acid-inhibitable manner.

1. Introduction

Nitric oxide (NO) is an important mediator of the inhibitory non-adrenergic non-cholinergic (NANC) neurotransmission in the gastrointestinal tract (Sanders and Ward, 1992; Lefebvre, 1995; Rand, Li, 1995). We also showed that NO is involved in NANC nerve-mediated relaxation in mouse gastric fundus (Ergün and Öğülener, 2001). It is well known that the nitrergic neurotransmitter has to diffuse from its neuronal site of synthesis to the target enzyme soluble guanylate cyclase in the neighboring effector smooth muscle cells. During this transit the nitrergic neurotransmitter is vulnerable to reactive oxygen species (ROS) attack such as superoxide anion (O_2^-), hydrogen peroxide and NO scavenging activities. Superoxide anion generators and free radical scavengers have shown to have an inhibitory effect on relaxation induced by NANC in different nitrergically-innervated mouse gastric fundal smooth muscle (Ögülener et al., 2006).

Flavonoids are polyphenolic compounds widely distributed in dietary fruits, vegetables, wine, and exhibit a wide range of biological effects such as reducing low-density lipoproteins in plasma, inhibiting platelet aggregation, scavenging free radicals, preventing cell proliferation, antibacterial, anti-inflammatory and antiallergic actions (Formica and Regelson, 1995; Cook and Samman, 1996; Middleton et al., 2000). Flavonoids have been also described to have relaxing effects on the contractility of various smooth muscle, such as vascular smooth muscle (Chan et al., 2000; Ajay et al., 2003; Morello et al., 2006), bladder (Dambros et al., 2005), vas deferens (Capasso and Mascolo, 2003) and stomach (Amira et al., 2008). Among dietary flavonols quercetin is by far the most abundant polyphenolic compounds found in the human diet. The beneficial effects of quercetin have been attributed to multiple mechanisms including antioxidant activity, anti-inflammation, modification of signal transduction pathways, and interaction with receptors and other proteins. However, most investigators accept that the biological action of quercetin is considered to be connected with its antioxidant properties which are mainly due to (1) its ability to scavenge free

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http://dx.doi.org/10.1016/j.ejphar.2012.10.009

radicals and ROS (superoxide anion, hydroxyl radical) (Afanas'ev et al., 1989; Bors et al., 1994; Jovanovic et al., 1994) and (2) to form complexes with metal ions, thus preventing oxidation of the metals with oxygen yielding ROS (Morel et al., 1994; Mira et al., 2002). However, there are also some findings contradicting the antioxidant features of quercetin, and suggesting that quercetin also scavenges nitric oxide (NO), accompanied by superoxide anion production depending on the physiologic conditions such as pH, O_2^- concentration and superoxide anion concentration (Vanacker et al., 1995; Haenen and Bast, 1999). It was shown that quercetin is a better scavenger of O_2^- than of NO in under conditions of increased O_2^- in vascular smooth muscle (López-López et al., 2004). Also, we reported that quercetin acts as a protective agent in mouse corpus cavernosum, increasing the bioavailability of exogenous NO by protecting it from superoxide anion (Ertuğ et al., 2010).

In this study, we aimed to compare the preventing capacity of quercetin with enzymatic antioxidant Cu/Zn SOD and non-enzymatic antioxidants, ascorbic acid and glutathione on NO-induced relaxation in mouse gastric fundus. For this purpose, we studied: (1) the effects of quercetin on nitrergic stimulation- and NO-induced relaxations in the presence of diethyldithiocarbamic acid (DETCA), an extracellular and intracellular superoxide dismutase inhibitor, pyrogallol and hydroquinone, an extracellular superoxide anion generator and LY83583, an extracellular and intracellular anion generator, and (2) the preventing capacity of quercetin compared with other antioxidants ascorbic acid, glutathione and Cu/Zn SOD on nitrergic stimulation and NO-induced relaxation in mouse gastric fundus. Furthermore, evaluation of the impact of the quercetin on the total oxidant and antioxidant capacity of tissue was also performed in the present study.

2. Materials and methods

2.1. Preparation of smooth muscle strips

Swiss albino mice of either sex, weighing 20–25 g, were use in these experiments. They were fasted for 24 h with free access to water. They were killed by stunning and cervical dislocation. The stomach was carefully removed and the fundus was isolated. Approximately 10 mm long and 2 mm wide strips were prepared by longitudinal incision and oriented between two platinum wire electrodes in an (10 ml) organ bath filled with Tyrode solution (in mM: NaCI 136.75, KCI 2.68, $CaCl_2$ 1.80, $MgCl_2 \cdot 6H_2O$ 0.95, $NaH_2PO_4 \cdot 2H_2O$ 0.4166, $NaHCO_3$ 11.904, glucose 5.05). The Tyrode solution always contained 1 μM atropine and 1 μM guanethidine to inhibit cholinergic and adrenergic responses. The bath medium was maintained at 37 °C and gassed with a mixture of 95% O_2 and 5% CO_2 at pH 7.4. Muscle strips were allowed to equilibrate for a period of 60 min, during which the medium was changed every 15 min. Over the first 30 min of incubation, the strips were stretched to obtain an initial tension of 0.5 g and kept at this level (0.5 g) throughout the experiments. It was not necessary to raise tone with exogenous contractile agonist in order to observe relaxant responses (Ergün and Öğülener, 2001; Öğülener and Ergün, 2002, 2004). Changes in muscle length were recorded isometrically via an isometric transducer (Ugo Basile 7006, Varese, Italy) connected to an ink-writer (Ugo Basile "Gemini" 7070, Varese, Italy). The local Ethics Committee of the University of Cukurova approved all experiments.

2.2. Experimental protocols

Once a stable basal tone was obtained, two series control relaxant responses were obtained according to the experimental protocol in mouse fundus preparations. In the experimental protocol, nitrergic stimulation (4 Hz, 25 V, 0.1 ms, 10 s-train), NO (10 μM; administered as acidified $NaNO_2$) and isoproterenol (0.005 μM) was applied at 3-min intervals without rinsing the tissue between each individual application to a single tissue. All the stimuli used to induce relaxation were performed in the same tissue. After the relaxant responses had been obtained, the tissues were washed out and incubated with the drug under study and second series of responses were recorded in the same manner. At the end of the experimental protocol SNP (10 μM) was added to the bath medium to achieve maximal relaxation.

In the first set of experiments, the effect of the quercetin (0.01–100 μM) was studied per se on the nitrergic stimulation (4 Hz, 25 V, 0.1 ms, 10 s-train), exogenous NO (10 μM) and isoproterenol (0.005 μM). After the relaxant responses had been obtained by nitrergic stimulation, exogenous NO and isopropterenol, the tissues were washed out and incubated with quercetin for 30 min, and second series of responses were recorded in the same manner. Also, the effects of ascorbic acid (500 μM), glutathione (100 μM) and exogenous Cu^{2+}/Zn^{2+} superoxide dismutase (SOD; 200 U/ml) were investigated per se on relaxant responses to nitrergic stimulation, exogenous NO and isoproterenol.

In a second set of experiments, we studied the effect of quercetin on the nitrergic relaxations in the present of superoxide anion generators. After the first responses to nitrergic stimulation, exogenous NO and isoproterenol were obtained, the mouse gastric fundus was treated for 30 min with the superoxide anion generators, pyrogallol (10 μM), hydroquinone (100 μM) and 6-anilino-5 .8-quinolinedione (LY83583; 5 μM) and then nitrergic stimulation, NO and isoproterenol were applied second time. The influence of quercetin (0.1 μM) and the other antioxidants, ascorbic acid (500 μM), glutathione (100 μM) and exogenous Cu^{2+}/Zn^{2+} superoxide dismutase (SOD; 200 U/ml) was studied on the inhibitory effects of pyrogallol, hydroquinone and LY-83583 .

In a third series of experiments, the NO protecting effect of quercetin was studied on relaxations induced by nitrergic stimulation, exogenous NO and isoproterenol in the presence of diethyldithiocarbamic acid (DETCA; 8 mM), the irreversibly inhibitor of extra- and intracellular Cu/Zn SOD. After the first control responses were obtained, DETCA was added to the medium, and relaxant stimuli were applied for the second time. The tissue was incubated with DETCA for 30 min. To study the influence of quercetin (0.1 μM), ascorbic acid (500 μM), glutathione (100 μM) and exogenous Cu/Zn SOD (200 U/ml) on the inhibitory effect of DETCA, the antioxidants were administered before relaxant responses were obtained in the presence of DETCA. The influence of quercetin, ascorbic acid, glutathione and Cu/Zn SOD in the presence of DETCA was studied in tissues from different animals. Also, we studied the effects of quercetin and antioxidants on the relaxation to DETCA (8 mM) plus superoxide anion generators, pyrogallol (10 μM), hydroquinone (100 μM) and 6-anilino-5 .8-quinolinedione (LY83583; 5 μM) in mouse gastric fundus.

2.3. Tissue samples for biochemical analysis

The isolated preparations were incubated in the organ baths under the same conditions as in tension recording experiments (Tyrode solution at 25 °C under a stream of 5% CO_2 and 95% O_2). The tissues were exposed to drugs for 30 min or without treatment, served as control. Tissues were subsequently blotted on filter paper, weighed and frozen by liquid nitrogen and stored in −20 C until processing. Tissues were diluted 1:9 with phosphate solution (50 mM, pH 7.4), then they were homogenized and centrifuged (Hettich, Centrifuge Mikro 220R, Germany) at 7000g for 10 min (4 °C). The supernatants were used to evaluate the