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RESEARCH ARTICLE

Mitigating role of quercetin against sodium fluoride-induced oxidative stress in the rat brain

Seyed Fazel Nabavi[1,2], Seyed Mohammad Nabavi[1,2], Ali Mohammad Latifi[1], Morteza Mirzaei[1], Solomon Habtemariam[3], and Akbar Hajizadeh Moghaddam[4]

[1]Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, 19945-546, Tehran, Iran, [2]National Elites Foundation of Iran, 14174, Tehran, Iran, [3]Pharmacognosy Research Laboratories, Medway School of Science, The University of Greenwich, Kent, ME4 4TB, UK, and [4]Department of Biology, University of Mazandaran, 47416-95447, Babolsar, Iran

Abstract

Context: Quercetin is a well known aglycone flavonoid that is widely found in different food sources.

Objective: In this study, the *in vivo* neuroprotective potential of quercetin against sodium fluoride-induced oxidative stress was evaluated.

Materials and methods: Wistar rats were divided into five treatment groups and then subjected to daily intraperitoneally treatment with quercetin (at 10 and 20 mg/kg body weight), vitamin C (at 10 mg/kg), or vehicle. After a 1 week treatment period, all groups except saline treated (normal group), were intoxicated with sodium fluoride (NaF) for 1 week. Rat brains were then removed and homogenized for measurement of antioxidant markers including superoxide dismutase (SOD), reduced glutathione, catalase, and lipid peroxidation final products.

Results: The thiobarbituric acid reactive substances (TBARS) levels in the heart homogenate of sodium fluoride treated rats (42.04 ± 2.14 nmol MDA eq/g tissue) increased compared to the normal rats (35.99 ± 1.08 nmol MDA eq/g tissue). Animals which were pretreated with quercetin at 20 mg/kg for 1 week prior to sodium fluoride intoxication showed significant reduction in the TBARS level (36.13 ± 1.12 nmol MDA eq/g tissue). Also, pretreatment with quercetin (20 mg/kg) restored the SOD and catalase activities and modified the level of reduced glutathione compared with the control group ($p > 0.05$).

Discussion and conclusion: The present study revealed a potent neuroprotective potential of quercetin against NaF-induced toxicity.

Keywords: Antioxidant enzymes, flavonoid, neural tissues, reduced glutathione

Introduction

Fluoride plays an important role in diminishing tooth decay and prevention of dental diseases. Recent studies, however, demonstrated that fluoride intoxication for extended period may cause oxidative injuries called "fluorosis". It is serious problem in some developing areas. Fluoride intoxication may happen through drinking water, food or industrial sources and from the fluoride abuse in dental care materials. Chronic fluoride intoxication or fluorosis has been shown to be associated with a manifestation of neurological dysfunctions in humans and experimental animals (Shivarajashankara et al., 2001). It has also been demonstrated that one of the various mechanisms of fluoride-induced injury of the central nervous systems is attributed to oxidative stress in neural cells (Inkielewicz & Czanowski, 2008). Moreover, reports from various laboratories established a close correlation between oxidative stress in animals and fluoride intoxication (Bhatnagar et al., 2002; Shivashankara et al., 2002). Antioxidants are thus likely to offer protective effect in fluoride induced damage in brain and other tissues.

Address for Correspondence: Seyed Mohammad Nabavi, Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, P.O. Box 19945-546, Tehran, Iran. Tel./Fax: +98 21 22823734. E-mail: Nabavi208@gmail.com

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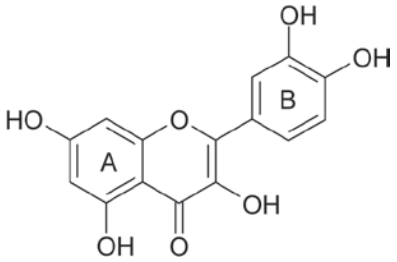

Figure 1. Chemical structure of quercetin.

Quercetin (Figure 1) is one of the most abundant flavonoids found in plants and foods (Heim et al., 2002). As a polyphenolic compound and with structural features well suited for metal chelation (Figure 1), quercetin is a potent reactive oxygen species scavenger and inhibitor of lipid peroxidation both *in vitro* and *in vivo* (Fiorani et al., 2001). Although pharmacokinetic and bioavailability knowledge of this flavonoid is scarce and contradictory, the compound has been shown to display various other biological activities including anticancer, antiulcer, antiallergic, anti-inflammatory, antiviral, antiproliferative, and cardioprotective effects (Rice-Evans et al., 1997).

To the best of our knowledge, the therapeutic potential of quercetin against fluorosis brain damage has not been investigated. The present study was thus designed to assess the effect of quercetin on antioxidant defense markers in rat brain following induction of oxidative stress by NaF.

Materials and methods

Chemicals

Bovine serum albumin (BSA) and a protein estimation kits were purchased from Ziest-Shimi Company (Tehran). Quercetin, 5,5-dithiobis(2-nitrobenzoic acid) [DTNB, Ellman's reagent], glacial acetic acid, heparin, nitro blue tetrazolium chloride (NBT), potassium dihydrogen phosphate (KH_2PO_4), reduced glutathione (GSH), sodium dihydrogen phosphate (NaH_2PO_4), sodium fluoride (NaF), trichloroacetic acid (TCA), thiobarbituric acid (TBA), and hydrogen peroxide were bought from Sigma-Aldrich Chemical Company (St. Louis, MO). Other chemical reagents were of analytical grade or purer.

Animals

The study was performed with 8–12-week-old male Wistar rats of body weight between 200–250 g. Animals were housed in ventilated animal rooms at a temperature of 24 ± 2°C with a 12 h light/dark cycle and 60 ± 5% humidity. A standard laboratory feed manufactured by Pasture Institute, Tehran, Iran was used while water was provided *ad libitum*. Experiments were performed between 10:00 and 14:00 h. Animals were kept for 1 week to acclimatize before starting the study. All experiments were performed according to the norms of the ethical committee of University of Mazandaran, Babolsar which follows the national guidelines for animal care and use (Approval number: No. S-2009 UMZ).

Animal treatments

Experimental animals were randomly divided into five groups of 10 animals each. Group I was kept as normal control receiving sample vehicle (0.5 mL, i.p.); Groups II and III were treated with the quercetin (10 and 20 mg/kg body weight, respectively), and IV group was treated with the vitamin C (10 mg/kg body weight). The treatment method was intraperitoneal route for 7 consecutive days. Except for the normal control group (Group I), all groups were treated with NaF (600 ppm through drinking water) for the next 7 days. Animals of Group V, which did not receive drug pretreatment, were used as a NaF (600 ppm through drinking water)-treated control group.

Anesthesia and tissues collect

At the end of the experiment, rats were anesthetized with intraperitoneally administration of ketamine (60 mg/kg) and xylazine (5 mg/kg). Rat's brains were removed. Brain tissues were kept at –60°C before biochemical studies.

Preparation of tissue homogenate

The whole brain tissue was homogenized in KH_2PO_4 buffer (100 mM, pH 7.4) containing 1 mM of EDTA (1:10 w/v) and centrifuged at 12000*g* for 30 min at 4°C. The supernatant was collect and used for biochemical measurements.

Determination of protein content

The protein content was determined by the method of Bradford (1976) using BSA as the standard.

Biochemical estimation

Estimation of lipid peroxidation

Lipid peroxidation as a measure of thiobarbituric acid reactive substances (TBARS) formation was determined by the method of Esterbauer and Cheeseman (1990). Tissue homogenates containing 1 mg protein was mixed with TCA (1 mL, 20%), thiobarbituric acid (2 mL, 0.67%). After incubation for 1 h at 100°C and cooling (room temperature), the precipitate was removed by centrifugation. The absorbance of the supernatant was then measured at 532 nm using a blank containing all the reagents except tissue homogenates.

Determination of superoxide dismutase activity

The reaction mixtures contained sodium carbonate (1 mL, 50 mM), nitroblue tetrazolium (0.4 mL, 25 μM) and freshly prepared hydroxylamine hydrochloride (0.2 mL, 0.1 mM). All test preparations were mixed by inversion followed by addition of clear supernatant of tissue homogenates (0.1 mL, 1:10 w/v). The change in absorbance of samples was recorded at 560 nm (Nabavi et al., 2012).

Table 1. Effect of pretreatment on superoxide dismutase and catalase activities and reduced glutathione, TBARS levels in sodium fluoride-induced oxidative stress in rat brain.

Group	SOD (U/mg protein)	Catalase (μmol/min/mg protein)	GSH (μg/mg protein)	TBARS (nmol MDA eq/g tissue)
Quercetin 20 + NaF	126.47 ± 5.06[c]	91.45 ± 3.96[c]	11.95 ± 0.41[c]	36.13 ± 1.12[c]
Quercetin 10 + NaF	92.88 ± 3.14[b]	83.4 ± 3.16[d]	9.55 ± 0.37[b]	38.89 ± 1.56[c]
Vitamin C + NaF	115.5 ± 4.97[b]	89.16 ± 3.48[c]	10.2 ± 0.39[b]	37.07 ± 1.26[c]
Normal rat	137.06 ± 5.22	94.71 ± 4.32	12.6 ± 0.45	35.99 ± 1.08
NaF	53.24 ± 1.62[b]	70.73 ± 2.94[b]	6.74 ± 0.31[b]	42.04 ± 2.14[a]

Values are mean ± standard deviation ($n = 10$). Data for normal animals are considered as base-line data; there was no significant base-line difference between the groups.
GSH, glutathione; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances
[a]$p < 0.01$ versus normal group.
[b]$p < 0.001$ versus normal group.
[c]$p > 0.05$ versus normal group.
[d]$p < 0.05$ versus normal group.

Determination of catalase activity

The catalase activity of brain tissues were determined spectrophotometrically according the method of Bonaventura et al. (1972). One unit of catalase activity is defined as the amount of enzyme that reduces 1 μmol of hydrogen peroxide/min.

Determination of reduced glutathione activity

The concentration of GSH (μM/g wet tissues) in brain tissues were estimated by evaluating free-SH groups, using 5,5′-dithiobis(2-nitrobenzoic acid) method described by Ellman (1959).

Statistical analysis

All data are presented as means and standard deviations (SD). Differences between group means were analyzed using a one-way analysis of variance followed by Duncan's multiple range tests. Results were considered statistically significant when $p < 0.05$.

Results

The level of TBARS in all treatment groups of rat brain homogenates are shown in Table 1. Lipid peroxidation in rat brain tissues homogenates from NaF-treated animal were significantly ($p < 0.01$, 42.04 ± 2.14 nmol MDA eq/g tissue) higher than the normal untreated group (35.99 ± 1.08 nmol MDA eq/g tissue). Animals which were pretreated with the positive control, vitamin C, for 1 week prior to NaF intoxication showed reduced levels of TBARS. As shown in Table 1, administration of quercetin prior to NaF intoxication showed a dose-dependent reduction in lipid peroxidation as assessed from TBARS measurements. While the reduction in catalase activity by NaF intoxication was marginal (though significant), the suppression of superoxide activity was dramatic with the enzyme activity ameliorated by 2.57-fold (versus the control group). The dose-dependent neuroprotection of quercetin was evident in these enzyme assays and the positive control; vitamin C did also show a good protective effect (Table 1). The reduced glutathione levels in rat brain tissue homogenates of different groups were also assessed. It is apparent from Table 1 that intoxication by NaF-induced a significant reduction (about two-fold) in the level of GSH in rat brain tissue homogenates. Once again, quercetin pretreatment caused a dose-dependent improvement in the level of reduced glutathione in rat brain. A good level of protection was also recorded for vitamin C when it was administered 1 week prior to NaF intoxication (Table 1).

Discussion

Some previous reports have highlighted the protective effect of quercetin against dexamethasone (Tongjaroenbuangam et al., 2011), methylmercury-induced (Wagner et al., 2010) and 6-hydroxydopamine-induced neurotoxicity (Haleagrahara et al., 2011). The aim of the present study was to evaluate if the preventive role of quercetin could be extended to NaF-induced oxidative stress in rat brain. In order to get a comprehensive set of results, four key oxidative parameters were evaluated in our study: TBARS levels, superoxide dismutase (SOD) and catalase enzyme activities and level of the reduced glutathione in rat brains. Our data unequivocally revealed that NaF administration induced significant changes in key oxidative biochemical parameters of rat brain homogenates. The validation of the methodology came through the use of a known antioxidant, vitamin C, which improved all the four biochemical parameters studied. Interestingly, intraperitoneal administration of quercetin before intoxication with NaF resulted in a dose-dependent improvement of antioxidant defenses. At the dose 20 mg/kg, quercetin totally reversed the NaF-mediated suppression of the studied antioxidant defenses

The *in vivo* generation of reactive oxygen species including superoxide anion, hydrogen peroxide and hydroxyl radical is known to be mediated by a variety of drugs, environmental toxicants and other pro-oxidative mechanisms (Matés et al., 1999). Antioxidant defenses such as SOD, catalase and reduced level of GSH are responsible for continuously keeping the level of reactive oxygen species checked. Thus, excessive levels of reactive oxygen species generation and/or diminished level of antioxidant defenses can lead oxidative stress. Under these circumstances, pro-oxidative damage to

macromolecular (e.g., DNA, proteins and lipids) and other cellular components are inevitable (Gul et al., 2000; Halliwell & Gutteridge, 2007). There is now overwhelming evidence to suggest that oxidative stress is linked to disease conditions including diabetes and neurodegenerative diseases (Yamagishi et al., 2001). Boosting antioxidant enzyme defenses (e.g., superoxide diusmutase and catalase) and GSH to maintain the normal physiological processes is thus paramount in combating these disease conditions (Gul et al., 2000). A number of antioxidant defenses such as vitamin C that come in the form of small molecular weight food components also play a key role in keeping the normal pro-oxidant-antioxidant balance. Furthermore, other natural products such as curcumin have been shown to ameliorate oxidative stress conditions induced by a variety of agents including, NaF (Nabavi et al., 2012).

Against all this background, the therapeutic potential of one the most abundant flavonoids, quercetin, for diseases associated with oxidative stress in the brain need to be evaluated. Our study established that, pretreatment of rats with quercetin at doses of 10 and 20 mg/kg body weight, prior to NaF-induced oxidative stress, can lead to improvement of antioxidants enzymes activity (i.e., SOD and catalase), increase the level of GSH and reduction in lipid peroxidation in rats brains.

Cholbi et al. (1991) demonstrated that hydroxyl groups of A ring (Figure 1) participate in the lipid peroxidation inhibition, but hydroxyls of the B ring are not important, though they enhanced the effects. Pretreatment with vitamin C also shows similar activity like the quercetin pretreated rats whereas a sample solvent has no useful activity. Our study therefore provides direct evidence in support of the therapeutic potential of quercetin in oxidative stress diseases in the brain.

Conclusion

The present study clearly demonstrated the neuroprotective effect of a flavonoid aglycone, quercetin, against NaF-induced oxidative stress in rat brains. Given that quercetin is an abundant natural product found in vegetables and edibles fruits, further clinical studies on its therapeutic potential is merited. Further studies on long-term toxicological profile of quercetin are also needed.

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This article is dedicated to Seyed Maryam Nabavi and with memory of Seyed Ali Asghar Nabavi.

Declaration of interest

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