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Enhanced neuroprotective effect of fish oil in combination with quercetin against 3-nitropropionic acid induced oxidative stress in rat brain

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ABSTRACT

While the beneficial effects of fish oil (FO) supplements on the central nervous system function are well established, few findings have led to the hypothesis that long term n–3 polyunsaturated fatty acid (n−3 PUFA) supplements at higher doses render the membranes more susceptible to lipid peroxidation. Hence recent studies suggest the use of dietary antioxidants as adjuncts with n−3 fatty acids to effectively improve the clinical outcome in neurological disorders. In the present investigation, we examined the hypothesis, if enrichment of FO with quercetin (a natural flavonoid) can provide a higher degree of neuroprotection and tested the same in a 3-nitropropionic acid (NPA) rat model. Growing male rats administered with NPA (25 mg/kg bw/d, i.p. 4 days) were provided either with FO (2 mL/kg bw), or Q (25 mg/kg bw) or FO + Q for 14 days. NPA elicited marked oxidative stress in brain (striatum and cerebellum) as evidenced by significantly enhanced ROS, malondialdehyde, protein carbonyls and nitric oxide levels. Although varying degree of protection was evident among FO or Q groups, complete normalization of oxidative markers ensued only among FO + Q rats. Further, FO + Q combination completely normalized the elevated acetylcholinesterase activity and protected against NPA-induced mitochondrial dysfunctions. NPA induced depletion of dopamine levels was restored among all groups. Interestingly, NPA induced motor deficits were significantly improved among FO + Q rats. However, further studies are necessary to understand the mechanism/s by which FO enrichment with Q provides higher degree of protection. Nevertheless, our findings clearly suggest that the use of natural phytochemicals with moderate doses of FO may provide better neuroprotection and higher therapeutic advantage in the prevention or treatment of neurodegenerative diseases like Huntington's disease.

1. Introduction

Fish oil (FO) provides a host of health benefits because of its major n−3 polyunsaturated fatty acid (n−3 PUFA) components: eicosapentaenoic acid (EPA, 20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3). Adequate dietary intake of PUFAs and in particular life-long DHA bioavailability provides considerable visual, neurovascular, cardiovascular, and neurological health benefits. EPA and DHA are vital for the normal, homeostatic operation of the central nervous system (CNS) (Palacios–Pelaez et al., 2010). The benefits of n−3 PUFA supplementation have been previously reported in various neurological disorders and experimental models of Alzheimer's and Parkinson's disease (Bousquet et al., 2009; Cole et al., 2009; Hashimoto et al., 2005). Recently, we reported the efficacy of FO supplements (2–4 mL/kg bw, 30 days) in modulating endogenous oxidative markers in rat brain. Further, FO supplements among growing rats significantly protected against rotenone-induced oxidative stress and mitochondrial dysfunctions (Denny Joseph and Muralidhara, 2012a).

Numerous studies demonstrate the beneficial effects of FO supplements, while a body of evidence highlights that dietary n–3 PUFA at 'high doses' and 'long duration' renders the membrane more susceptible to lipid peroxidation (Tsuduki et al., 2011). Hence, recently attempts have been made to enhance the neuroprotective efficacy of FO by fortification with antioxidants/phytochemicals (Mazza et al., 2007). Supplementation of FO with astaxanthin increased the antioxidative capacity in brain homogenates, reduced lipid peroxidation and protein oxidation in anterior forebrain of rats (Mattei et al., 2011). In a transgenic AD mice model, combined supplementation of FO with curcumin or epigallocatechin-3-gallate (EGCG) was found to be beneficial (Giunta et al., 2010; Ma et al., 2009). Supplementation of DHA with lutein significantly improved the memory scores and rate of learning in older women (Johnson et al., 2008).

Quercetin (Q), one of the most widely distributed flavonoids in plants, possesses free radical scavenging properties, protects the cells from oxidative injury and promotes cell survival (Bournival et al., 2009; Kahraman et al., 2012). Q is a more potent antioxidant

Abbreviations: ROS, reactive oxygen species; Q, quercetin; NPA, 3 nitropropionic acid; MDA, malondialdehyde; DCF, 2′,7′–dichlorofluorescein; DCFH–DA, 2′,7′–dichlorofluorescein diacetate; n−3 PUFAs, n−3 polyunsaturated fatty acids; FO, fish oil; NO, nitric oxide.

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than other antioxidant nutrients, such as vitamin C, vitamin E, and β-carotene (Mishra and Flora, 2008). Several experimental studies showed the potential of Q to offset cognitive deficit in various animal models (Bhutada et al., 2010). Q increases mitochondrial biogenesis in brain, which may have important implications on neurodegenerative conditions where mitochondrial dysfunctions play a major role (Davis et al., 2009). A recent study showed the propensity of Q to ameliorate oxidative stress-induced neuronal cell death in a 6-OHDA model of Parkinson's disease in rats (Sriraksa et al., 2012). Since FO and Q act on different pathways, the combined supplementation may significantly enhance the beneficial effects. Previously, incorporation of quercitrin (glycosylated from of Q) into FO was shown to synergistically enhance the anti-inflammatory effects of FO in induced intestinal colitis (Camuesco et al., 2006). Further, Q incorporation into n–3 enriched surimi gels enhanced the antioxidant capacity of the gel and when fed for three weeks, these gels significantly increased the total serum antioxidant capacity in rats (Perez–Mateos et al., 2005). In this regard, our recent findings suggest that fish oil in combination with Q markedly attenuated oxidative stress/mitochondrial dysfunctions in a rotenone model of neurotoxicity in rats (Denny Joseph and Muralidhara, 2012b).

3-Nitropropionic acid (NPA), a well known fungal toxin causes significant neurotoxicity in both animals and humans. Administration of NPA to rats induces neuronal damage that mainly affects the striatum and mimics many of the histological and neurochemical features of Huntington's disease (HD) (Tunez et al., 2010). The mechanism of NPA induced neurotoxicity involves inhibition of succinate dehydrogenase (SDH), an enzyme that acts in tricarboxylic acid cycle and the electron transport chain at complex II (Herrera–Mundo and Sitges, 2010). Recent studies clearly demonstrate that increased oxidative stress is one of the major deleterious events in NPA-induced neurodegenerative process. Neuronal damage caused by NPA can be attenuated by various compounds including phytochemicals (Shinomol and Muralidhara, 2008; Shinomol et al., 2012).

In the present study, we tested the hypothesis that enrichment of quercetin (a natural flavonoid) with a moderate dose of FO (2 mL/kg bw for short duration) is likely to provide better neuroprotection employing a NPA rat model. The neuroprotective efficacy of FO + Q combination was assessed in terms of its potency to attenuate NPA induced oxidative stress in cytosol/mitochondria, mitochondrial dysfunctions and locomotor deficits.

2. Materials and methods

2.1. Chemicals

Thiobarbituric acid (TBA), quercetin (Q), 1,1,3,3-tetramethoxy propane, 2′,7′-dichlorofluorescein (DCF), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), and 3-nitropropionic acid (NPA) were purchased from M/s Sigma Chemical Co. St. Louis, USA. All other chemicals used were of analytical grade.

2.2. Animals and care

Growing male Wistar rats (4 week-old) drawn from the stock colony of our animal house facility were housed in rectangular polypropylene cages kept on racks built of slotted angles, in a controlled atmosphere with a 12 h light/dark cycle. They were acclimatized for 3 days prior to the start of the experiment. The animals were maintained on a commercial powdered diet and tap water *ad libtum*. The experiments were conducted strictly in accordance with approved guidelines by the "Institute Animal Ethical Committee" regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social justice and Empowerment, Government of India, India.

2.3. Fish oil (FO): composition and dosages

Cod liver oil purchased from Seven seas (Seacod) was used throughout the study and GC analysis showed that it contained 7.5% EPA and 6.5% DHA. In the present study, we used FO at a dosage of 2 mL/kg bw/d for 14 days. Selection of this dosage is based on our preliminary study (Denny Joseph and Muralidhara, 2012a). In this study, we employed a lower dosage of FO for short duration to determine the combined effect of FO with Q.

2.4. Quercetin: dosage selection

Selection of Q dosage was based on published literature and preliminary standardization from our laboratory. Similar dosages of Q have been employed by various workers (Camargo et al., 2011; Davis et al., 2009; Naidu et al., 2003).

2.5. Experimental design

NPA was dissolved in physiological saline and intraperitoneally (i.p.) administered to rats based on body weight. FO, quercetin (Q) and the combination of FO + Q were given as oral supplements. Q was dissolved in 1% polyethylene glycol (PEG). Oral supplements were given 1 h prior to NPA administration on first four consecutive days. From day 5 onwards, only the supplements were continued until day 14. The dosage of NPA used in this study was based on previous literature (Sandhir et al., 2010), and preliminary studies from our laboratory.

Rats were randomly assigned to five groups of six animals (n = 6) each and treated as follows:

Group 1 (vehicle control) — rats given physiological saline;
Group II NPA control — rats administered with NPA (i.p., 25 mg/kg bw) for the first 4 days;
Group III FO + NPA: NPA administered rats given FO (2 mL/kg bw) supplements for 14 days;
Group IV Q + NPA: NPA administered rats given Q (25 mg/kg bw) supplements for 14 days; and
Group V FO + Q + NPA: NPA administered rats given FO + Q supplements for 14 days.

Behavioral assessments were carried out on day 7 and 14. Rats of both control and treatment groups were necropsied on day 15, brain was excised and brain regions viz., cerebellum and striatum were dissected over ice. From each region, cytosol and mitochondrial fractions were prepared and subjected to quantification of various biochemical parameters.

2.6. Behavioral assessments

2.6.1. Narrow beam test

Narrow beam test was used to measure hind-limb impairments as described by Sandhir et al. (2010) with minor modifications. Animals were trained to traverse a 150 cm long wooden beam, divided into three 50 segments (1, 2, 3) from a platform at one end to the animal's home cage at the other end, placed horizontally 60 cm above the floor. Each rat was tested 3 times and the scores were given as follows: a score of 1 for a rat that traverses the beam without falling; a score of 2 if it fell off in the third segment; a score of 3 if it fell in second segment; 4 if it fell in first segment and 5 if the rat failed to even balance/sit down on the beam. The average scores of three trials per rat were taken.

2.6.2. Landing foot spread distance (LFSD)

LFSD was measured among both control and treated rats as described previously (Pradat et al., 2001). In this behavior test, the fourth digit of the hindlimbs of the rat is colored with ink and the