Biochimica et Biophysica Acta 1832 (2013) 421–430

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Quercetin supplementation is effective in improving mitochondrial dysfunctions induced by 3-nitropropionic acid: Implications in Huntington's disease

Rajat Sandhir *, Arpit Mehrotra

Department of Biochemistry, Basic Medical Science Building, Panjab University, Chandigarh, 160014, India

ARTICLE INFO

Article history:
Received 3 March 2012
Received in revised form 21 November 2012
Accepted 27 November 2012
Available online 4 December 2012

Keywords:
Flavonoid
Huntington's disease
Mitochondrion
Neurodegeneration
3-Nitropropionic acid
Quercetin

ABSTRACT

The study was designed to investigate the beneficial effect of quercetin supplementation in 3-nitropropionic acid (3-NP) induced model of Huntington's disease (HD). HD was induced in rats by administering sub-chronic dose of 3-NP, intraperitoneally, twice daily for 17 days. Quercetin was supplemented at a dose of 25 mg/kg body weight by oral gavage for 21 days. At the end of treatment, mitochondrial bioenergetics, mitochondrial swelling, oxidative stress, neurobehavioral deficits and histopathological changes were analyzed. Quercetin supplementation was able to reverse 3-NP induced inhibition of respiratory chain complexes, restore ATP levels, attenuate mitochondrial oxidative stress in terms of lipid peroxidation and prevent mitochondrial swelling. Quercetin administration also restored the activities of superoxide dismutase and catalase along with thiol content in 3-NP treated animals. Beneficial effect of quercetin administration was observed on 3-NP induced motor deficits analyzed by narrow beam walk and footprint analysis. Histopathological analysis of 3-NP treated rats revealed pyknotic nuclei and astrogliosis in striatum, which were reduced or absent in quercetin supplemented animals. Altogether, our results show that quercetin supplementation to 3-NP induced HD animals ameliorated mitochondrial dysfunctions, oxidative stress and neurobehavioral deficits in rats showing potential of this flavonoid in maintaining mitochondrial functions, suggesting a putative role of quercetin in HD management.

1. Introduction

Huntington's disease (HD) is a progressive, fatal, neurodegenerative disorder caused by an expanded CAG repeat in the huntingtin gene (Htt), that encodes for an abnormally long polyglutamine tract in a protein termed huntingtin (htt) with a molecular weight of approximately 350 kDa [1]. The disease is inherited in an autosomal dominant manner with age-dependent penetrance. Clinical features of HD include progressive motor dysfunctions, cognitive decline, and psychiatric disturbances, including both neuronal dysfunctions and neuronal cell death [2]. Although, mutation in Htt gene was discovered more than 17 years ago, the role of Htt in the physiology and the pathophysiology is still under investigation [3]. Recent data indicates that the translocation of mHtt into nucleus and transcriptional dysregulation likely play an important role in the pathogenic process and more specifically these events have a significant impact on mitochondrial functions such as electron transport chain (ETC) and reactive oxygen species (ROS) generation leading to bioenergetic failure [4]. Numerous studies in cell and mouse models of HD have revealed mitochondrial impairment [5].

The hypothesis that mitochondrial dysfunctions contribute to the pathogenesis of HD was first tested pharmacologically by using 3-nitropropionic acid (3-NP), an irreversible inhibitor of succinate dehydrogenase [6]. One of the mechanisms following 3-NP administration is the development of mitochondrial dysfunctions leading to generation of a bioenergetic defect which involves three interacting processes such as: energy impairment, oxidative stress and excitotoxicity [7]. 3-NP induced HD model replicates most of the clinical and pathophysiological hallmarks of HD, including spontaneous choreiform and dystonic movements, frontal-type cognitive deficits, and progressive striatal neuronal degeneration [8]. 3-NP administration also results in ATP depletion, which impairs intracellular calcium buffering thereby leading to production of damaging ROS [9].

At present, there are no effective treatments against HD. Current therapies for treating HD are symptomatic; focusing on neurological and psychiatric symptoms that aim at improving the quality of life [10]. Attention has been given on the influence of phytochemical therapeutics on health and mental well-being. Evidence has indicated that a group of plant-derived compounds known as flavonoids exerts particularly powerful action as cardioprotective, chemopreventive and neuroprotective agents [11]. The biological activities of flavonoids have been attributed to their antioxidant, anti-inflammatory and their property to modulate signaling cascades [12]. Within the flavonoid family, quercetin is the most potent scavenger of ROS and

Abbreviations: 3-NP, 3-nitropropionic acid; CAT, catalase; DTNB, 5,5-dithiobis-2-nitrobenzoic acid; ETC, electron transport chain; HD, Huntington's disease; LMW-SH, low molecular weight thiols; MDA, malondialdehyde; NBT, nitroblue tetrazolium; PSH, protein thiols; ROS/RNS, reactive oxygen and nitrogen species; SOD, superoxide dismutase; SDH, succinate dehydrogenase; TSH, total thiols

* Corresponding author at: Department of Biochemistry, Panjab University, Chandigarh, 160014, India. Tel.: +91 172 2534131/38.
E-mail address: sandhir@pu.ac.in (R. Sandhir).

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http://dx.doi.org/10.1016/j.bbadis.2012.11.018

reactive nitrogen species (RNS). This can be explained by the presence of two antioxidant pharmacophores within the molecule that have the optimal configuration for free radical scavenging [13]. Quercetin has been shown to easily traverse the blood–brain-barrier and acts as promising agents for intervention in neurodegenerative conditions like ischemia [14], Alzheimer's disease [15] and Parkinson's disease [16]. Use of neuroprotective antioxidants is being considered as a promising approach to slow down the disease progression and to limit the extent of functional neuronal loss in chronic neurodegeneration as well as after acute lesions of the brain. However, only a few studies on the use of antioxidants in the management of neurodegenerative conditions have so far been undertaken. Methodological inconsistencies, poor permeation of the blood–brain barrier and lower efficacy per dose are some reasons for the lack of studies in this area [17]. Quercetin exerts its beneficial effect in brain primarily through its antioxidant action. Quercetin has been shown to improve mitochondrial functions in brain by increasing mitochondrial biogenesis [18]. Within the subcellular compartment quercetin shows preferential accumulation in mitochondria [19]. Based on the information in the literature, quercetin appears to be promising agent against HD. Therefore, in the present study, we have evaluated the neuroprotective potential of quercetin against 3-NP induced mitochondrial oxidative stress, mitochondrial dysfunctions and neurobehavioral deficits.

2. Experimental procedures

2.1. Chemicals

All the chemicals used in the present study were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, USA), Merck (Mumbai, India) and Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Quercetin was purchased from Himedia Laboratories Pvt. Ltd. (Mumbai, India).

2.2. Animals and treatment schedule

Female Wistar rats aged 9–10 weeks, weighing between 200 and 250 g were procured from the Central Animal House facility of Panjab University, Chandigarh, India. The animals were allowed to acclimatize to the local vivarium for 7 days. All the experiments were carried out between 09:00 and 15:00 h. The protocols followed were approved by the Institutional Animal Ethics Committee of the University and were in accordance with the guidelines for humane use and care of laboratory animals. The animals were randomly segregated into the following four groups with each group having 6–8 animals.

Control (vehicle): animals were given vehicle.
Control + quercetin treated: animals were administered with vehicle for 3-NP and quercetin at a dose of 25 mg/kg through oral gavage for 21 days.
3-NP treated: animals were administered 3-NP at a sub-chronic dose twice a day intraperitoneally for 17 days. Twice a day injections were: 7.5 mg/kg for the first 2 days, followed by 3.75 mg/kg for next 7 days, finally a dosage of 2 mg/kg for the last 8 days. The dose of 3-NP used in the study is based on the doses reported in literature and were standardized in our laboratory [20].
3-NP + quercetin treated: One hour before sub-chronic 3-NP treatment, animals were administered with quercetin at a dose of 25 mg/kg by oral gavage for 21 days.

2.3. Mitochondrial respiratory chain enzymes

2.3.1. Isolation of rat brain mitochondria

On day 21, animals were sacrificed by decapitation under mild ether anesthesia. Mitochondria were isolated from striatum by the method described by Puka-Sundvall [21]. Briefly, corpus striatum was dissected, rinsed in ice-cold isotonic saline and homogenized in ice-cold extraction buffer (10 mM Tris–HCl, pH 7.4, 0.44 M sucrose, 10 mM EDTA and 0.1% BSA). The homogenate was centrifuged at 2100 *g* for 15 min at 4 °C. The pellet was discarded and the supernatant re-centrifuged at 14,000 *g* for 15 min at 4 °C. The crude mitochondrial pellet was separated, washed with extraction buffer and centrifuged at 7000 *g* for 15 min at 4 °C. The final mitochondrial pellet was re-suspended in buffer containing 0.44 M sucrose in 10 mM Tris–HCl, pH 7.4.

2.3.2. NADH dehydrogenase (complex I)

Activity of NADH dehydrogenase was measured as described by King and Howard [22]. Requisite amount of mitochondrial preparation was added to the reaction mixture containing 0.2 M glycyl-glycine (pH 8.5), 6 mM NADH, 1 mM oxidized cytochrome c and 0.02 M $NaHCO_3$. NADH dehydrogenase catalyzed reduction of cytochrome c and the increase in absorbance was followed spectrophotometrically at 550 nm for 3 min. Results were expressed as nmol NADH oxidized/min/mg protein. The enzyme activity was normalized to citrate synthase activity.

2.3.3. Succinate dehydrogenase (complex II)

Activity of succinate dehydrogenase was assayed according to the method of King et al. [23]. The reaction mixture contained 0.2 M sodium phosphate buffer (pH 7.8), 1% (w/v) BSA, 0.6 M succinate and 0.03 M potassium ferricyanide. The reaction was initiated by addition of requisite amount of mitochondrial preparation. Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate by potassium ferricyanide, which was measured spectrophotometrically by decrease in absorbance at 420 nm for 3 min. Results were expressed as nmol succinate oxidized/min/mg protein. The enzyme activity was normalized to citrate synthase activity.

2.3.4. Cytochrome oxidase (complex IV)

Activity of cytochrome oxidase was assayed according to the method described by Sottocasa et al. [24]. Oxidized cytochrome c was reduced by adding few crystals of sodium borohydride and then neutralized to pH 7.0 by 0.1 M HCl. 0.3 mM of reduced cytochrome c was added to 0.075 M phosphate buffer and the reaction was initiated by mixing appropriate amount of mitochondrial suspension. The reduced cytochrome c is oxidized in the reaction mixture containing cytochrome oxidase which is measured spectrophotometrically by a decrease in absorbance at 550 nm for 3 min. Results were expressed as nmol cytochrome c oxidized/min/mg protein. The enzyme activity was normalized to citrate synthase activity.

2.3.5. F_1F_0 synthase (complex V)

Mitochondrial F_1F_0 synthase activity was measured as described by Griffiths and Houghton [25]. Reaction was started by adding appropriate amount of mitochondrial suspension in ATPase buffer [50 mM Tris and 5 mM $MgCl_2$, pH 7.5] at 37 °C with 5 mM ATP for 10 min. The reaction was stopped by adding 10% (w/v) trichloroacetic acid. The contents were centrifuged at 3000 *g* for 20 min, and an appropriate volume of supernatant was mixed with water. Phosphate produced was measured by the method of Fiske and Subbarow [26]. Results were expressed as nmol of ATP hydrolyzed/min/mg protein. The enzyme activity was normalized to citrate synthase activity.

2.3.6. MTT reduction

The reduction of MTT to blue formazan by dehydrogenases present in the mitochondrial suspension was also monitored to assess mitochondrial functions [27]. To appropriate mitochondrial pellet, MTT (0.1 mg/ml) was added, mixed and incubated at 37 °C for 30 min and then centrifuged to obtain formazan pellet. The pellet was dissolved in absolute ethanol and the mixture was re-centrifuged at 2000 *g* for 10 min. The absorbance of the supernatant was measured at 595 nm.