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BRAIN RESEARCH

Research Report

Lack of robust protective effect of quercetin in two types of 6-hydroxydopamine-induced parkinsonian models in rats and dopaminergic cell cultures

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ABSTRACT

In the present study, we examined the ability of a flavonoid quercetin to prevent 6-hydroxydopamine (6-OHDA)-induced oxygen radical formation and cytotoxicity in vitro and neurotoxicity in vivo. Quercetin (10–100 μM) had an acute significant antioxidant effect against the 6-OHDA-induced (30 μM) oxygen radical formation in catecholaminergic SH-SY5Y neuroblastoma cells. Moreover, in these cells, quercetin at 10–50 μM had a significant protective effect against 6-OHDA though at 100 μM it was itself harmful to the cells. The possible effect of quercetin in preventing neurotoxicity in unilateral medial forebrain bundle (full nigral lesion) or striatal (partial lesion) 6-OHDA rat lesion models of Parkinson's disease was studied in three treatment schedules: a 7-day pre- or post-treatment or their combination. Rotational responses to apomorphine (0.1 mg/kg, subcutaneously) and D-amphetamine (2.5 mg/kg, intraperitoneally) were assessed at weeks 1 and 2 post-lesion. Quercetin had no consistent neuroprotective effect in either model at 50–200 mg/kg once a day or 100 mg/kg twice a day. Furthermore, no protection was observed in tyrosine hydroxylase positive nigral cell numbers, striatal fiber density or in striatal levels of dopamine. These in vitro and in vivo results cast doubt on the theory that quercetin exerts reliable neuroprotective effects against 6-OHDA-induced toxicity. In vitro, quercetin seems to be protective at low doses but damaging at high doses.

1. Introduction

Parkinson's disease (PD) is characterized by a pronounced degeneration of dopaminergic neurons in the substantia nigra (SN) resulting in a reduction of the striatal dopamine levels. Several biochemical mechanisms have been proposed as playing critical roles in the pathogenesis of PD, one of which is the deleterious effects of oxidizing metabolites and free radicals (Fahn and Cohen, 1992; Jenner, 2003). The role of oxidative stress in PD has been studied extensively. Dopamine

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Abbreviations: 6-OHDA, 6-hydroxydopamine; PD, Parkinson's disease; SN, substantia nigra; ROS, reactive oxygen species; CMC, carboxymethylcellulose; MFB, medial forebrain bundle; TH, tyrosine hydroxylase; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid; LDH, lactate dehydrogenase; MTT, methyl tetrazolium

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itself is auto-oxidized to quinones and to reactive oxygen species (ROS), such as H_2O_2, superoxide anion ($O_2^{\cdot-}$) and hydroxyl radical ($OH^{\cdot}$) (Olanow, 1990). In the SN, oxidative stress may develop due to increased dopamine turnover, resulting in excess H_2O_2 formation (see Jenner and Olanow, 1996). Post-mortem studies in PD patients have provided evidence of chemical changes that are indicative of oxidative stress in the SN, including increased levels of lipid peroxidation, protein oxidation, 3-nitrotyrosine formation, DNA strand breaks and decreased levels of ROS scavenging enzymes such as glutathione peroxidase and catalase (see Jenner and Olanow, 1998).

Much of our knowledge about dopaminergic neurodegeneration has emerged from studies with the selective catecholaminergic neurotoxin, 6-hydroxydopamine (6-OHDA). 6-OHDA is selectively taken up by the plasma membrane dopamine transporter and subsequently accumulates in the mitochondria (Blum et al., 2001). 6-OHDA administration results in the formation of ROS. Intranigral and intrastriatal injections in rodents evoke a dramatic destruction of dopaminergic neurons in the SN pars compacta accompanied by a marked reduction of dopaminergic terminals in the striatum (Kirik et al., 1998; Kumar et al., 1995). The toxic effects of 6-OHDA have also been demonstrated in vitro in dopaminergic cell cultures (Puttonen et al., 2003). Since the neurotoxicity of 6-OHDA is evidently mediated by non-enzymatic oxidative stress and excessive generation of free radicals (Kumar et al., 1995), it could be speculated that the oxidative damage evoked by 6-OHDA should be prevented by antioxidants. In fact, compounds like melatonin and vitamin E, have been found to be effective against 6-OHDA-induced neurodegeneration in some experimental settings (Aguiar et al., 2002; Heim et al., 2001; Sharma et al., 2006).

In vitro studies have demonstrated some efficacy of bioflavonoids, tea extracts containing polyphenolic constituents and catechins in attenuating 6-OHDA-induced cell death in pheochromocytoma and neuroblastoma cells (Nobre Junior et al., 2003). Flavonoids are phenolic plant compounds that occur widely in nature and possess potent antioxidant and iron chelating properties (Mira et al., 2002). One such compound, quercetin (3,5,7,3′,4′-pentahydroxyflavone), and its metabolites have strong oxygen radical scavenging properties. It also inhibits xanthine oxidase and lipid peroxidation in vitro (Fiorani et al., 2001). Furthermore, quercetin has been found to act as an antioxidant in vitro in PC12 cells against H_2O_2-induced apoptosis (Dajas et al., 2003).

We are aware of one report exploring effects of quercetin on 6-OHDA-induced neurodegeneration in rat (Zbarsky et al., 2005). This report cast doubt on the neuroprotective efficacy of quercetin in vivo but the dose of quercetin used was rather small. Furthermore, in this case, 6-OHDA lesions were achieved by administration of toxin into medial forebrain bundle (MFB), which rapidly evokes a near complete striatal dopamine depletion in lesioned side (Yuan et al., 2005). On the other hand, repeated administration of quercetin has shown beneficial effects in other types of animal models involving neurotoxicity (Naidu et al., 2003; Patil et al., 2003; Singh et al., 2003). Finally, quercetin has been claimed to cause apoptosis (Wei et al., 1994) and to act as an anti-tumour agent (Levy et al., 1984). To solve these controversies and to reveal the true importance of quercetin as a neuroprotective compound, we have now examined the cytoprotective effect of quercetin in in vitro studies with catecholaminergic SH-SY5Y neuroblastoma cell cultures and assessed comprehensively the neuroprotective effect of various quercetin treatment schedules (pre-and post-treatment and their combination) against 6-OHDA-induced neurotoxicity in vivo using two types of unilateral 6-OHDA-lesion rat models of PD. In the full lesion model, a high dose of 6-OHDA was infused into the MFB leading to a severe dopaminergic damage, and in the partial lesion model, small doses of 6-OHDA were administered into four sites of the striatum offering more potential for the neuroprotective treatment. In addition, we performed some immunohistochemical and biochemical studies to supplement our behavioral results.

2. Results

2.1. *The effect of quercetin on 6-OHDA toxicity in SH-SY5Y cell cultures (MTT and LDH)*

The effect of quercetin on 6-OHDA-induced neurodegeneration in SH-SY5Y neuroblastoma cell line was assayed in two ways: 1) using an index of metabolic activity (MTT assay) and 2) assessing cell membrane integrity (LDH release). 6-OHDA

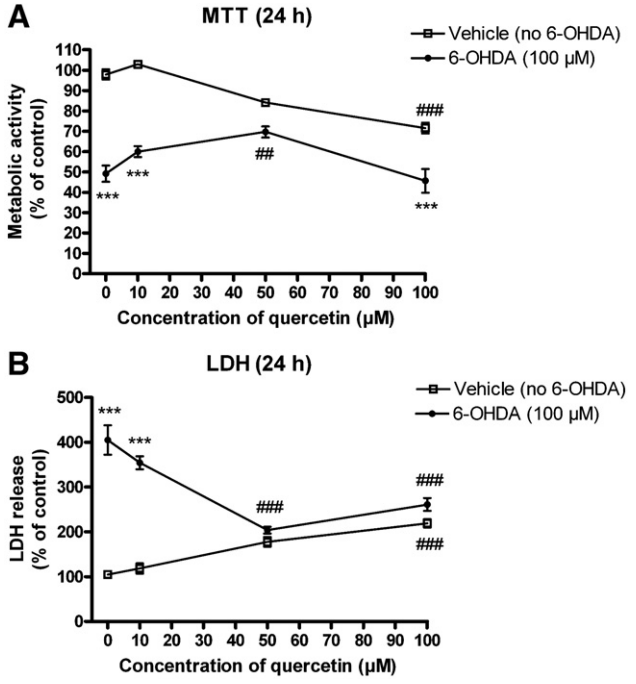

Fig. 1 – The effect of quercetin alone (vehicle, no 6-OHDA) and with 6-OHDA (100 μM) on toxicity in SH-SY5Y cell cultures. Cell viability of SH-SY5Y cells was quantified by MTT reduction assay (A). Cell death was quantified by the release of LDH from SH-SY5Y cells into the medium (B). Various concentrations of quercetin were added to the cells 1 h prior to vehicle (containing 0.1% DMSO) or the 6-OHDA exposure and incubated further for 24 h. Data represents the mean ± SEM of 4 independent experiments, each carried out with 4 replicates. $^{***}p<0.001$ 6-OHDA versus vehicle at the respective concentration of quercetin, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ quercetin versus respective control (0 μM of quercetin).