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Mechanism of the beneficial effect of melatonin in experimental Parkinson's disease

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ABSTRACT

This study aimed to elucidate locomotor activity changes in 6-hydroxydopamine (6-OHDA) induced Parkinson's disease (PD) and investigate the possible beneficial effects of melatonin on altered levels of locomotor activity, cyclooxygenase (COX), prostaglandin E2 (PGE2), nuclear factor kappa-B (NF-κB), nitrate/nitrite and apoptosis. Male Wistar rats were divided into five groups: vehicle (V), melatonin-treated (M), 6-OHDA-injected (6-OHDA), 6-OHDA-injected + melatonin-treated (6-OHDA-Mel) and melatonin treated + 6-OHDA-injected (Mel-6-OHDA). Melatonin was administered intraperitoneally at a dose of 10 mg/kg/day for 30 days in M and Mel-6-OHDA groups, for 7 days in 6-OHDA-Mel group. Experimental PD was created stereotactically via unilateral infusion of 6-OHDA into the medial forebrain bundle (MFB). The 6-OHDA-Mel group started receiving melatonin when experimental PD was created and treatment was continued for 7 days (post-treatment). In the Mel-6-OHDA group, experimental PD was created on the 23rd day of melatonin treatment and continued for the remaining 7 days (pre- and post-treatment). Locomotor activity performance decreased in 6-OHDA group compared with vehicle; however melatonin treatment did not improve this impairment. Nuclear factor kappa Bp65 and Bcl-2 levels were significantly decreased while COX, PGE2 and caspase-3 activity were significantly increased in 6-OHDA group. Melatonin treatment significantly decreased COX, PGE2 and caspase-3 activity, increased Bcl-2 and had no effect on NF-κB levels in experimental PD. 6-Hydroxydopamine injection caused an obvious reduction in TH positive dopaminergic neuron viability as determined by immunohistochemistry. Melatonin supplementation decreased dopaminergic neuron death in 6-OHDA-Mel and Mel-6-OHDA groups compared with 6-OHDA group. Melatonin also protected against 6-OHDA-induced apoptosis, as identified by increment in Bcl-2 levels in dopaminergic neurons. The protective effect of melatonin was more prominent for most parameter following 30 days treatment (pre- and post-) than 7 days post-treatment. In summary, melatonin treatment decreased dopaminergic neuron death in experimental PD model by increasing Bcl-2 protein level and decreasing caspase-3 activity.

1. Introduction

Melatonin is a natural hormone synthesized and released by the pineal gland (Niranjan et al., 2010). Melatonin is an extremely potent free-radical scavenger and antioxidant. Its protective and anti-inflammatory effect in numerous neurological disorders including Parkinson's disease (PD) have been previously demonstrated (Lin et al., 2013; Niranjan et al., 2010; Patki and Lau, 2011).

Parkinson's disease is the second most common neurodegenerative disease that affects about 1.8% of old people over 65 years of age. Parkinson's disease is characterized by progressive loss of dopaminergic neurons in the substantia nigra (SN) and depletion of dopamine (DA) in the corpus striatum (Hornykiewicz and Kish, 1987). The etiology of PD is not completely clear yet, however several hypotheses to explain neuronal death has been put forward, such as; mitochondrial dysfunction, oxidative stress, exitotoxicity and inflammation (Dauer and Przedborski, 2003; Moore et al., 2005). In line with these hypotheses, there are several studies indicating oxidative stress in the pathology of PD (Adams et al., 2001; Przedborski, 2005). Since reactive oxygen species (ROS) are accumulated more in dopaminergic neurons, it makes these neurons more vulnerable to neural degeneration (Graham, 1978). As a result

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of enzymatic metabolism of DA, hydrogen peroxide (H_2O_2) and superoxide radicals (O_2^-) are formed, also when the auto-oxidation product of DA combines with iron (Fe^{2+}), it leads to the formation of DA-quinones that causes the formation of hydroxyl radicals (·OH) (Ben-Shachar et al., 1991; Graham, 1978). Furthermore, the synthesis of DA by tyrosine hydroxylase (TH) and catabolism by monoamine oxidase (MAO) can result in the formation of H_2O_2 (Andersen et al., 1994; Graham, 1984). Reactive oxygen species have been detected in the SN of Parkinsonian patients and experimental animal models (Przedborski, 2005). Mitochondria which is the main source of ROS, contributes to the pathogenesis of PD (Przedborski, 2005).

In many studies, when compared with the control groups of the same age, lipid peroxidation was proven to be higher in the SN of patients with PD (Dexter et al., 1989; Yoritaka et al., 1996). Because of these reasons, systems that produce oxidative stress are very important in the pathology of PD.

In addition to oxidative stress, the amount of neuronal nitric oxide synthase (nNOS) and inducible nitric oxide synthase (iNOS) are increased in PD (Aras et al., 2014; Levecque et al., 2003). Together with this increase, peroxynitrite ($ONOO^-$) is also formed (Chabrier et al., 1999). It is known that nitric oxide (NO) is a free radical and forms nitrogen derivatives such as nitrites and nitrates in the presence of oxygen. It has been detected that oxygen radicals formed as a result of auto-oxidation react with NO to form $ONOO^-$ (Beckman and Koppenol, 1996). It has also been demonstrated that $ONOO^-$ is a strong oxidant that can destroy several biologic molecules and independent of metal catalysis dissociates to form ·OH (Beckman and Koppenol, 1996). Nitric oxide also affects nuclear factor kappa-B (NF-κB) activation to cause apoptosis (Pannu and Singh, 2006).

It has been demonstrated that 6-OHDA causes the activation COX-2 and increases the level of mRNA (Jin et al., 2008; Pyo et al., 2013). It is also known that the amount of PGE2 metabolite is increased in tissues as an indicator of high level of COX-2 (Esposito et al., 2007; Jin et al., 2008; Lee et al., 2010; Ozsoy et al., 2011; Pyo et al., 2013). Lipid peroxidation is known to increase as a result of all these events.

Conducted studies demonstrated that melatonin decreases 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced lipid peroxidation *in-vivo* (Thomas and Mohanakumar, 2004) and caspase-3 enzyme activity *in-vitro* (Sharma et al., 2005). Attempts have been made to explain the mechanism of this process using Fe^{2+}, the Haber–Weiss reaction and glial cell line-derived neurotrophic factor (GDNF) (Ortiz et al., 2013). However the effect of melatonin on 6-OHDA induced lipid peroxidation and caspase-3 enzyme activity is not known. It has been demonstrated in separate studies that melatonin inhibits iNOS and COX-2 activity (Absi et al., 2000; Dabbeni-Sala et al., 2001; Vilar et al., 2014). Its protective effects could be explained by the oxidative enzymes in mitochondria (Dastgheib and Moezi, 2014; Raghavendra and Kulkarni, 1999). It has been shown in microglia cell line studies that melatonin inhibits iNOS (Tocharus et al., 2008). Additionally, it has been detected that it protects neurons by inhibiting iNOS (Cardinali et al., 2013). Furthermore, it has been detected that melatonin increases the amount of anti-apoptotic Bcl-2 and decreases the amount of pro-apoptotic Bax protein (Ma et al., 2009). It is also known that death of dopaminergic neurons containing TH decreased by the administration of melatonin in experimental PD (Grealish et al., 2008; Sharma et al., 2007). However, there is no study in the literature concerning the effect of melatonin on COX, PGE2, NF-κB, iNOS and Bcl-2 in 6-OHDA lesion model of PD.

This study was performed to clarify the anti-apoptotic mechanism of melatonin in 6-OHDA-induced hemiparkinsonian rat model. The role of COX, PGE2, NF-κB, iNOS, caspase-3 and Bcl-2 on this effect was evaluated. Our study is important in terms of demonstrating the protective effect of melatonin in the progression of 6-OHDA model of PD.

2. Materials and methods

2.1. Animals

Male Wistar rats (3 month old, weighing 250–300 g) were obtained from Akdeniz University Animal Care Unit. The animals were housed in stainless steel cages (5–6 per cage) in an air-conditioned room (22 ± 2 °C with a 12:12 h light:dark cycle). All experimental protocols conducted on rats were performed in accordance with the standards established by the Institutional Animal Care and Use Committee at Akdeniz University Medical School.

2.2. Experimental design

Rats were randomly divided into five experimental groups as follows: Vehicle (V) ($n = 40$); melatonin-treated (M) ($n = 40$); 6-OHDA-injected (6-OHDA) ($n = 40$); 6-OHDA-injected + melatonin-treated (6-OHDA-Mel) ($n = 40$) and melatonin-treated + 6-OHDA-injected (Mel-6-OHDA) ($n = 40$) group.

2.3. Surgery

Lesion surgery was conducted under chloral hydrate (Merck, Darmstadt, Germany) (400 mg/kg, ip.) anesthesia in a stereotaxic frame. 6-Hydroxydopamine hydrochloride (6-OHDA-HCl) (Sigma, Steinheim, Germany) was dissolved in 1% ascorbate (Sigma) saline. For injection, a burr hole was drilled above the injection site and the tip of the syringe lowered into the target. The right medial forebrain bundle (MFB) was lesioned by injection of freshly prepared, 12 μg (freebase) of 6-OHDA-HCl (3×4 μg/μl solution at 1 μl/min rate) (Hacioglu et al., 2012) at stereotaxic coordinates AP –2.2, ML ±1.5 (from bregma) and DV –8.0 below dura (Kayir et al., 2009; Paxinos et al., 1985). After injection the cannula was left in place for three minutes before slowly retracting it. Finally, the skin incision was sutured. All three groups of animals continued to receive their usual treatments for additional days until sacrificed.

2.4. Melatonin administration

Melatonin (Sigma, St. Louis, MO, USA) was dissolved in absolute ethanol and diluted in normal saline. The ethanol concentration in the final solution was 10%. Melatonin was adjusted to a dose of 10 mg/kg/day and intraperitoneally injected for 30 days in M group. The 6-OHDA-Mel group started receiving melatonin when experimental PD was created and the treatment was continued for 7 days until sacrification. In Mel-6-OHDA group, experimental PD was created on the 23rd day of melatonin administration and melatonin treatment was continued for the remaining 7 days until sacrification. The same volume of 10% ethanol was administered to the vehicle treated animals (group V and 6-OHDA).

2.5. Locomotor activity test

Seven days after injection of 6-OHDA, locomotor activity was measured with an open-field activity monitoring system (MAY 9908