β-Naphthoflavone, Ethanol Reverse Mitochondrial Dysfunction

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The 1-methyl-4-phenylpyridinium (MPP^+) is a parkinsonian-inducing toxin that promotes neurodegeneration of dopaminergic cells by directly targeting complex I of mitochondria. Recently, it was reported that some Cytochrome P450 (CYP) isoforms, such as CYP 2D6 or 2E1, may be involved in the development of this neurodegenerative disease. In order to study a possible role for CYP induction in neurorepair, we designed an in vitro model where undifferentiated neuroblastoma SH-SY5Y cells were treated with the CYP inducers β-naphthoflavone (βNF) and ethanol (EtOH) before and during exposure to the parkinsonian neurotoxin, MPP^+. The toxic effect of MPP^+ in cell viability was rescued with both βNF and EtOH treatments. We also report that this was due to a decrease in reactive oxygen species (ROS) production, restoration of mitochondrial fusion kinetics, and mitochondrial membrane potential. These treatments also protected complex I activity against the inhibitory effects caused by MPP^+, suggesting a possible neuroprotective role for CYP inducers. These results bring new insights into the possible role of CYP isoenzymes in xenobiotic clearance and central nervous system homeostasis.

Keywords: Cytochrome P-450 System ; neuroprotection ; CYP induction

1. Introduction

Degeneration of dopaminergic neurons in the substantia nigra is a main feature of Parkinson's disease (PD). Genetic and environmental factors are known to give rise to differential proteostatic states in the brain. Additionally, cell specific energy metabolism defects and reactive oxygen species (ROS) production can enhance neurodegeneration rates[1]. ROS can also be generated by exposure to environmental xenobiotics or drug metabolism. In addition, genetic predisposition contributes to selective neurodegeneration of these cells[2][3][4][5]. High oxidative stress and disruptors of mitochondrial membrane potential (ψ_m) lead to a change in morphology and alteration in mitochondrial fusion-fission dynamics[6]. These events disrupt the overall mitochondrial homeostasis, promoting apoptosis, the release of pro-apoptotic factors, and contributing to neurodegeneration in PD and other diseases[7][8]. In this context, xenobiotics have emerged as one important source of oxidative stress that may lead to mitochondrial dysfunction in the brain[8][9].

2. Mechanism

A mechanism by which the cells clear xenobiotics is by the Cytochrome P-450 system (CYP), which metabolize a wide variety of molecules[10]. In the brain, CYP represents a 0.5–2% of the total amount of CYP found in the liver, but it still plays an important role in the metabolism of drugs and some endogenous compounds in the central nervous system (CNS)[11]. This superfamily has several isoforms with specific expression patterns depending on the brain area and the cell type[12][13]. In particular, the isoform CYP 2D6 has been related with the development of PD due to its ability to metabolize several xenobiotics in dopaminergic cells and other areas[14]. Moreover, in dopaminergic cells, the induction of CYP 2E1 by nicotine or coffee has been related with less susceptibility to PD[15][16]. However, the contributions of CYPs isoforms to neurodegeneration and neuroprotection toward xenobiotic insult are still poorly understood.

Induction of CYP isoforms have been generally used for the study of drug metabolism and neuroprotection in vivo and in vitro[11]. Ethanol (EtOH) and β-naphthoflavone (βNF) are two well-known inducers of CYP isoforms[17][18][19]. We previously reported that both compounds promote the induction of CYP 2D6 and 2E1, and that CYP 2D6 can be localized in mitochondria in SH-SY5Y cells[20]. The objective of the present study is to elucidate whether treatments with both inducers protect mitochondria towards the neurotoxic effect of MPP^+. Our results suggest that, in parallel with induction of CYP isoforms 2D6 and 2E1, the two compounds reverse the mitochondrial impairment promoted by MPP^+.

Exposure to xenobiotics is one of the major causes of oxidative stress and apoptosis in the CNS and increases the risk of developing neurodegenerative diseases such as PD [21]. A mechanism by which the cells eliminate xenobiotics is the CYP system, which is involved in the metabolism of the drugs and toxins that cross the blood-brain barrier. Among the several isoforms that can be found in this super-family, most of them can be upregulated by at least a few xenobiotics[11]. In our previous publication, we demonstrated that βNF and EtOH are able to induce the expression of two isoforms in SH-SY5Y cells, CYP 2D6 and 2E1[20]. However, the mechanisms by which CYP isoforms can influence the overall homeostasis of the brain are poorly understood. In this study, we used SH-SY5Y cells to induce the expression of CYPs prior to exposure to MPP^+, with the aim to study how these two isoforms protect mitochondria against MPP^+ toxicity. Other publication has also shown that the apoptosis caused by MPP^+ is mediated by ROS production[22]. We showed that both βNF and EtOH treatments rescue the decrease in cell viability promoted by MPP^+. Additionally, we presented evidence that the observed neuroprotection is linked to a reduction of ROS formation, restoration of ψ_m and mitochondrial fusion kinetics. Finally, we showed that the toxic effect is avoided before MPP^+ affects the mitochondrial complex I activity. Taken together, these results suggest that induction of CYPs by βNF and EtOH may contribute to neuroprotection of SH-SY5Y against MPP^+ toxicity; however, other molecular mechanisms involving neuroprotection pathways not related with CYPs may not be discarded.

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