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Evaluation of antiparkinson activity of PTUPB by measuring dopamine and its metabolites in Drosophila melanogaster: LC–MS/MS method development

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ABSTRACT

Soluble epoxide hydrolase (sEH) inhibition is reported to elevate endogenous epoxyeicosatrienoic acids (EET's), which are known to play an important role in neuroprotection by inhibiting oxidative stress and neuroinflammation. In the present study, PTUPB, a dual inhibitor of sEH and COX-2, has been tested for its antiparkinson activity against rotenone (ROT) induced neurodegeneration in *Drosophila* model of Parkinson's disease (PD). To determine the efficacy and brain bioavailability of PTUPB a simple, rapid and sensitive LC–MS/MS method was developed and validated for the estimation of PTUPB (Method-I), dopamine (DA) and its metabolites (Method-II) in fly head. Mass spectrometric acquisitions of analytes signals were performed in positive and negative electron spray ionization MRM mode by monitoring the daughter ions. The isocratic elution using formic acid (0.1% v/v) and acetonitrile (20:80 v/v) (for method I), and acetic acid (0.1% v/v) and methanol (for method II) on Jones C_{18} was carried out to achieve the separation. The results of brain PTUPB, DA and its metabolites estimation shows a dose dependent increase in PTUPB concentration and a dose dependent prevention of ROT induced changes in DA and its metabolites levels ($p < 0.05$), indicating a significant neuroprotection activity of PTUPB. In the present study, we have successfully developed and validated LC–MS/MS methods to identify and quantify PTUPB, DA and its metabolites using a UFLC-ESI-QqQ mass spectrometer for the screening of neuroprotective agents in *Drosophila Melanogaster*.

1. Introduction

Parkinson's disease (PD) is characterised by progressive loss of dopaminergic neurons in the nigrostriatal system of the brain which results in bradykinesia, rigidity, resting tremor and posture instability. The major pathological events leading to neurodegeneration includes increased ROS production resulting in oxidative stress, neuroinflammation and apoptosis [1–3]. This in turn results in the imbalance in dopamine (DA) synthesis and metabolism (Fig. 1). The arachidonic acid (AA) released from membrane phospholipids by phospholipase A2 (PLA2) is metabolized to prostaglandins and thromboxane by cyclooxygenase (COX), to leukotrienes by lipoxygenase (LOX), and to epoxyeicosatrienoic acid (EETs) by cytochrome P450 (CYP450) oxidases [4,5]. The EETs are quickly metabolized to inactive or less active metabolites by soluble epoxide hydroxylases (sEH). EETs' are reported

Abbreviations: PD, Parkinson disease; DA, Dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, Homovanillic acid; BBB, blood brain barrier; sEH, soluble epoxide hydrolase; mEH, microsomal epoxide hydroxylases; COX-2, cyclooxygenase-2; ROS, reactive oxygen species; AA, arachidonic acid; EETs, Epoxyeicosatrienoic acids; LOX, lipoxygenase; PLA2, phospholipase A2; CYP450, cytochrome P450; ROT, rotenone; IS, internal standard; ACN, acetonitrile; CON, control; CPCSEA, Control and Supervision of Experiments on Animals; UGC, University Grants Commission; MCI, Medical Council of India.

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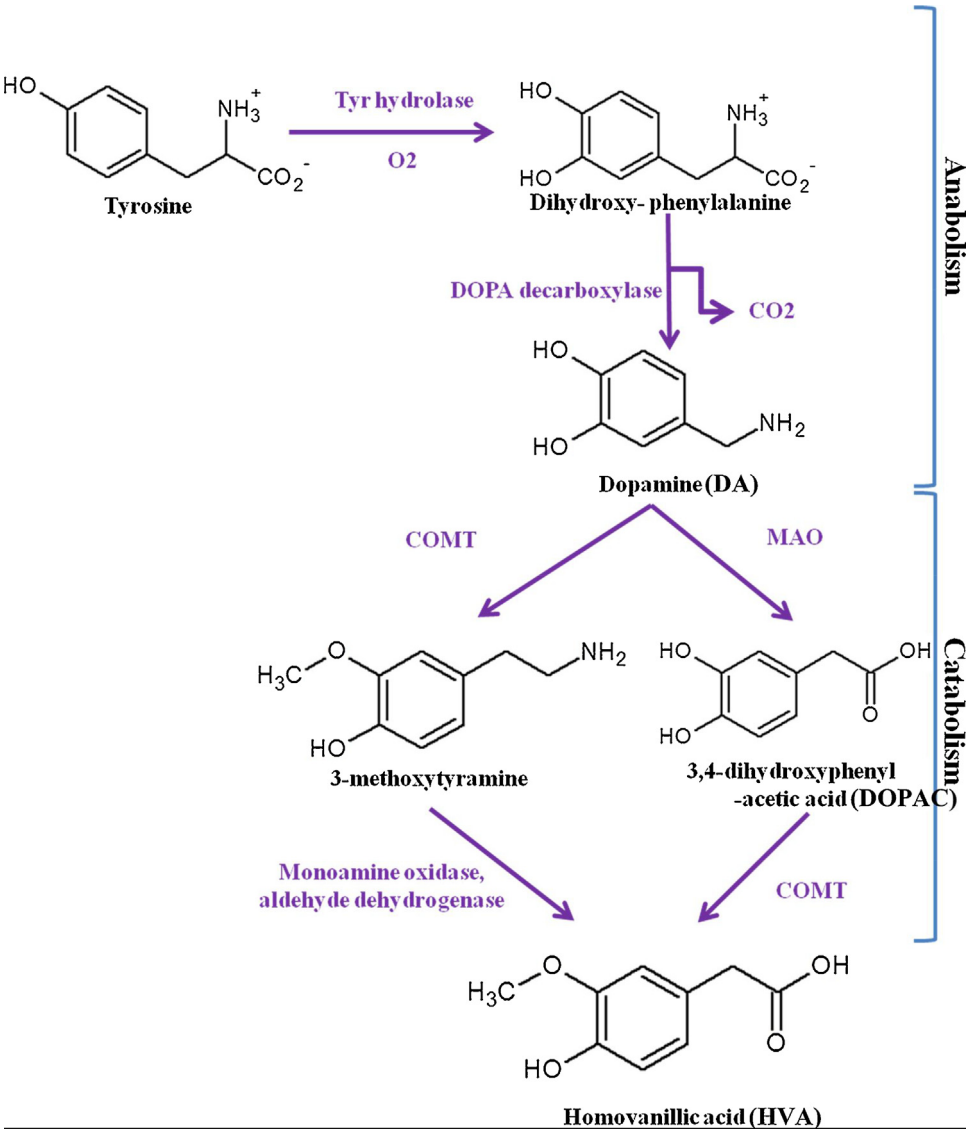

Fig. 1. Synthesis and metabolism of Dopamine (Abbreviations: MAO- Monoamine oxidase, COMT- catechol-o-methyltransferase).

to play a vital role in cytoprotection, due to their ability to attenuate oxidative stress, inflammation, and apoptosis [3]. It has been previously reported that progressive neuroinflammation induces the release of COX-2 which is rapidly expressed in several cell types in response to cytokines, and pro-inflammatory mediators [6]. One of the novel strategies, therefore, is simultaneous inhibition of both sEH and COX-2 enzymes. Since the pathogenesis in PD is multifactorial, involving mitochondrial dysfunction, oxidative stress and inflammation, the novel compound (s) that simultaneously target multiple degenerate pathways are required [1]. PTUPB (4-(5-phenyl-3-3-3-(4-trifluoromethyl-phenyl)-ureido-propyl-pyrazol-1-yl)-benzenesulfonamide), a dual inhibitor of sEH and COX-2 is expected to stabilize the EETs and thereby promote its cytoprotective actions such as anti-oxidant, anti-inflammatory, and anti-apoptosis. The simultaneous inhibition of COX-2 by PTUPB is also expected to reduce neuroinflammation mediated cell death [1,7,8].

The flies such as *Drosophila melanogaster* have been employed in elucidating various pathological mechanisms of human neurodegenerative disorders. The *Drosophila* model has been widely studied in case of genomic, cellular and developmental knowledge and the flies display surprisingly intricate behaviours and have complicated brain and nervous systems [9]. The fly model, therefore, provides a well-characterised system that is relatively easy to manipulate but complex enough to be relevant to the development of human disease models [10,11]. The rotenone (ROT) induced mitochondrial dysfunction, oxidative stress and inflammation in *Drosophila* is a well-accepted model for PD, which has been widely exploited in screening of potential molecules [12–14]. The global animal testing regulations that permit and control the use of non-human animals for research vary greatly around the world, but most governments aim to control the number of times individual animals may be used; the overall numbers used; and the degree of pain that may be inflicted without using anesthetic. All the regulatory bodies in general recommend adopting the principles of 3 R's i.e., replacement, refinement and reduction [15,16]. In this changing scenario, development of alternatives are, therefore needed. A number of computer simulations, *in vitro* techniques and other alternative models have been recommended in the place of animals. The *Drosophila* model is considered as one of the appropriate replacement models for rodents and other higher animals used in PD research. The model is inexpensive to propagate, maintain and can produce a large number of genetically homogenous progeny [17]. The present study, aims to evaluate antiparkinson's activity