PLoS one

Identification by Virtual Screening and *In Vitro* Testing of Human DOPA Decarboxylase Inhibitors

Frederick Daidone[1,9], **Riccardo Montioli**[2,9], **Alessandro Paiardini**[1,9], **Barbara Cellini**[2], **Antonio Macchiarulo**[3], **Giorgio Giardina**[1], **Francesco Bossa**[1], **Carla Borri Voltattorni**[2]*

1 Department of Biochemical Sciences ''A. Rossi Fanelli'', University of Rome ''La Sapienza'', Rome, Italy, 2 Department of Life Sciences and Reproduction, University of Verona, Verona, Italy, 3 Department of Chemistry and Drug Technology, University of Perugia, Perugia, Italy

Abstract

Dopa decarboxylase (DDC), a pyridoxal 5'-phosphate (PLP) enzyme responsible for the biosynthesis of dopamine and serotonin, is involved in Parkinson's disease (PD). PD is a neurodegenerative disease mainly due to a progressive loss of dopamine-producing cells in the midbrain. Co-administration of L-Dopa with peripheral DDC inhibitors (carbidopa or benserazide) is the most effective symptomatic treatment for PD. Although carbidopa and trihydroxybenzylhydrazine (the *in vivo* hydrolysis product of benserazide) are both powerful irreversible DDC inhibitors, they are not selective because they irreversibly bind to free PLP and PLP-enzymes, thus inducing diverse side effects. Therefore, the main goals of this study were (a) to use virtual screening to identify potential human DDC inhibitors and (b) to evaluate the reliability of our virtual-screening (VS) protocol by experimentally testing the ''in vitro'' activity of selected molecules. Starting from the crystal structure of the DDC-carbidopa complex, a new VS protocol, integrating pharmacophore searches and molecular docking, was developed. Analysis of 15 selected compounds, obtained by filtering the public ZINC database, yielded two molecules that bind to the active site of human DDC and behave as competitive inhibitors with K_i values ≥ 10 µM. By performing *in silico* similarity search on the latter compounds followed by a substructure search using the core of the most active compound we identified several competitive inhibitors of human DDC with K_i values in the low micromolar range, unable to bind free PLP, and predicted to not cross the blood-brain barrier. The most potent inhibitor with a K_i value of 500 nM represents a new lead compound, targeting human DDC, that may be the basis for lead optimization in the development of new DDC inhibitors. To our knowledge, a similar approach has not been reported yet in the field of DDC inhibitors discovery.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: carla.borrivoltattorni@univr.it

9 These authors contributed equally to this work.

Introduction

Parkinson's disease (PD) is the most extensively studied pathology within a group of syndromes called ''motor system disorders'', whose etiology can be traced back to the loss of dopaminergic neurons of the *substantia nigra* in the midbrain [1]. Main symptoms of PD include tremors, rigidity, bradykinesia and postural instability; other frequently observed symptoms include depression and other psychiatric disorders, difficulty in swallowing, chewing, and speaking. Early symptoms of PD are usually subtle and occur gradually after 50 years of age. As the symptoms become more severe, patients progressively encounter difficulties in walking, talking, or even completing the simplest tasks; usually, this condition interferes strongly with most daily activities.

At present there is no cure for PD, but a variety of palliatives reducing the severity of disease symptoms exists [2]. In order to replenish dopamine levels at the central nervous system (CNS), L-Dopa is usually administered. The latter is converted to dopamine by Dopa decarboxylase (DDC, E.C. 4.1.1.28), a pyridoxal-5'-phosphate (PLP)-dependent enzyme, which is abundant in the

CNS and in the kidney [3]. DDC from pig kidney has been widely characterized with respect to reaction and substrate specificity [4,5], spectroscopic features of the internal aldimine and of enzyme-intermediate complexes [6,7,8], and the role played by residues at or near the active site in the catalysis [9,10,11,12]. Moreover, the crystal structures of DDC, both ligand-free and in complex with the antiParkinson drug carbidopa, have been solved [13].

Although administration of exogenous L-Dopa to PD patients compensates, at least transitorily, for deficiency of dopamine synthesis and often provides dramatic relief from the main symptoms, only 1–5% of L-Dopa reaches the dopaminergic neurons of the brain, being the major part metabolized by the peripheral DDC. Therefore, in order to increase the amount of L-Dopa in the CNS, DDC inhibitors unable to cross the blood-brain barrier (BBB) are usually co-administered with L-Dopa. In this way, not only greater amounts of L-Dopa can reach the brain, thereby substantially increasing its level, but also side effects, either dopamine-related or due to a high concentration of L-Dopa in the blood stream, are diminished [1]. The most commonly used DDC