Development and characterisation of a tyrosinase-based biosensor for the in vitro-study of a new class of natural-like tyrosinase inhibitors

G. Rocchitta[1], S. Cossu[1], M. Pirisinu[1], R. Migheli[1], G. Delogu[2], M. A. Dettori[2], D. Fabbri[2], P. A. Serra[1]

1 Dept. of Clinical and Experimental Medicine, Medical School, University of Sassari, Sassari, Italy.
2 Sassari Unit, Institute of Biomolecular Chemistry of CNR, Sassari, Italy.

Over the past years the enzyme tyrosinase (polyphenol oxidase, EC 1.14.18.1) has received considerable attention as an indispensable tool in the performance of studies on a wide range of topics. Tyrosinase catalyses three different reactions in the biosynthetic pathway of melanin in melanocytes: the hydroxylation of tyrosine to L-DOPA and the oxidation of the L-DOPA to dopaquinone. (Parvez et al 2007). Futhermore, in humans, dopaquinone is converted by a series of complex reactions involving cyclization and oxidative polymerizations which finally result in the formation of melanin (Olivares et al., 2001). However, this beneficial trait comes in hand with some severe vices and human diseases because of the overproduction of melanin (Fu et al 2005, Meada et al 1991, Mcevily et al 1992). As, the unregulated action of tyrosinase is factor in a number of human disease etiologies, Tyrosinase inhibition has thus been avidly explored as an avenue for therapies to these diseases. Besides, some authors suggested that tyrosinase may contribute to the neurodegeneration associated with Parkinson's disease (Xu et al 1997).

The aim of the present study is the development of new pharmacological tools for preliminary studies on the Tyrosinase enzyme inhibition role eventually in melanoma and Parkinosn's disease.

In this study, it was designed, synthesized and characterized a new class of low molecular weight phenols, related to chalcones and phenylpropanoid, and in particular reminiscent of natural structures, having inhibitory activity against tyrosinase.

A first approach consisted in the determination of the inhibitory activity of each molecule by means of an amperometric biosensor. This device exploits the ability of the Tyrosinase to catalytically transform catechols to quinones, which are electrochemically detected on the surface of epoxy carbon-based microsensors, suitably modified, by applying a reduction potential of -50 mV vs Ag/AgCl.

The interaction between inhibitor and enzyme could be revealed from the variations in the Michaelis-Menten kinetic parameters, extrapolated both in the presence and absence of the molecule inhibitor. The specific kind of inhibition could be identified by means of Lineweaver–Burk plot, matching results obtained with and without the Tyrosinase inhibitor.

Moreover, the effects of same molecules were preliminary tested on the viability of PC12 cells, a cell line derived by murine pheochromocytoma, (in culture with medium alone or supplemented with hydrogen peroxide) in order to test their eventual biological propreties.

References:

Parvez S, Kang M, Chung HS, Bae H. Phytother Res. 2007, 21(9), 805-16.
Olivares C, Jiménez-Cervantes C, Lozano JA, Solano F, García-Borrón JC. Biochem J. 2001, 354(Pt 1):131.
Fu, B.; Li, H.; Wang, X.; Lee, F. S. C.; Cui, S. J. Agric. Food Chem. 2005, 53, 7408.
Meada, K.; Fukuda, M. J. Soc. Cosmet. Chem. 1991, 42, 361.
Mcevily, J. A.; Iyengar, R.; Otwell, Q. S. Crit. Rev. Food Sci. Nutr. 1992, 32, 253.
Xu, Y.; Stokes, A. H.; Freeman, W. M.; Kumer, S. C.; Vogt, B. A.; Vrana, K. E. Mol. Brain Res. 1997, 45, 159.