17:20 Poster 121

CHO cell line with stable expression of the HTRA1 gene as a tool for studying functional activity of 5-HT$_{1A}$ receptor ligands

Jarosław Walory[1], Sylwia Rzońca[2], Andrzej J. Bojarski[3], Zdzislaw Chilmonczyk[1]

1. *National Medicines Institute (NIL), Chełmska 30/34, Warszawa 00-725, Poland* **2.** *Center of Oncology Maria Sklodowska Curie Memorial Institute, W.K. Roentgena 5, Warsaw 02-781, Poland* **3.** *Polish Academy of Sciences. Institute of Pharmacology, Smętna 12, Kraków 31-343, Poland*

e-mail: walory@il.waw.pl

In order to obtain the appropriate CHO (Chinese Hamster Ovary) cell line overexpressing 5-hydroxytryptamine 1A receptor gene (HTR1A gene, human cDNA clone ref. ID NM_000524) pcDNA 3.1 (+) and pCMV6-XL4/HTR1A plasmids were processed with *Not I* restrictive enzyme and ligated. The obtained pcDNA 3.1(+) vector containing *HTR1A* gene was propagated in competent *E. coli* cells on LB-agar plates containing ampicilin. Individual clones containing the pcDNA/HTR1A plasmid were picked up and cultured in the LB Broth medium.

After isolation the pcDNA/HTR1A plasmid has been transferred into CHO-K1 (clone-1)cells with the aid of FuGene reagent. Then the cells were cultured in the presence of geniticine. Selected single cells were cloned and the expression of pcDNA/HTR1A plasmid was evaluated with the aid of Western blot analysis.

The resulting clone CHO-K1 cell lines with stable overexpression of the gene *HTRA1* were used as a model for testing the functional activity of 5HT$_{1A}$ receptor ligands.

Acknowledgements

This study was supported by a grant PNRF-103-AI-1/07 from Norway through the Norwegian Financial Mechanism.

17:20 Poster 123

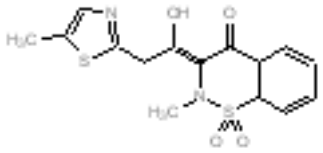
Effects of nonenzymatic glycosylation and fatty acids presence on meloxicam binding to human serum albumin

Katarzyna Wiglusz[1], Lilianna Trynda-Lemiesz[1,2], Marta Bałtrukiewicz[2], Halina Podsiadły[2]

1. *Wrocław Medical University, Faculty of Pharmacy, Department of Analytical Chemistry (AM), Szewska 38, Wrocław 50-139, Poland* **2.** *University of Wrocław, Faculty of Chemistry, Joliot-Curie 14, Wrocław 50-383, Poland*

e-mail: wiglusz@cheman.am.wroc.pl

One of the most important factors affecting the distribution and the active concentration of many administered drugs is binding affinity for human serum albumin (HSA). In the bloodstream and tissue fluids, HSA serves as a vehicle for the transport of several endogenous compounds including unesterified fatty acids (FA), hemin, bilirubin and thyroxine all of which bind with high affinity.



The structure of meloxicam

Long-chain fatty acids, a major source of cellular energy, are solubilized and transported in the blood by binding to serum albumin. Crystallographic studies of HSA have mapped at least seven FA binding sites and delineated the overlap with binding sites of some drugs and other endogenous compounds. The interactions of fatty acids with albumin modulates the ligand binding properties of protein by inducing conformational changes in the binding sites I and II, respectively. Thereby, understanding albumin fatty acid interactions is of major clinical and pharmaceutical importance.

In the circulation, HSA becomes nonenzymatically glycosylated by reducing sugars, and the reference range in normal humans is 6–10% glycoalbumin. However, this proportion typically increases to between 20% and 30% in hyperglycemic patients. The principal site of glycosylation of HSA is Lys-525, but the lysine residues in positions 199, 281 and 439 are also susceptible to modification. The effect of nonenzymatic glycosylation can influence on the affinity binding of drugs at site I (subdomain IIA) of albumin molecule.

Meloxicam is pharmacologically important new generation, non-steroidal anti-inflammatory drug (NSAID) of enolic acid class compounds with a minimum adverse gastrointestinal and renal side effects associated with traditional NSAID [1].

The primary function of meloxicam is anti-inflammatory effect but they can also be used as agents in cancer treatment, because in various types of cancer, cyclooxygenase-2 is over expressed. The nature of interaction of meloxicam with HSA involve strong drug–protein interactions with only high affinity site located in subdomain IIA [2,3].

The conformational changes associated with nonenzymatic glycosylation and FA binding may alter the physiological functions of albumin and binding of drugs.

In the present paper the influence of myristic acid and the effect of nonenzymatic glycosylation on meloxicam binding to the albumin using fluorescence quenching method and circular dichroism spectroscopic technique were investigated. The studies have shown that the FA and nonenzymatic glycosylation induce conformational changes in the albumin molecule. These changes affect on the binding ability of meloxicam towards albumin. The association constants in both cases were lower as compared to native protein.

1. G. Dannhardt, W. Kiefer, Eur. J. Med. Chem. 36 (2001) 109-126

2. N. Seedher, S. Bhatia, J. Pharm. Biomed. Anal. 39 (2005) 257-262

3. L. Trynda-Lemiesz, K.Wiglusz , J. Pharm. Biomed. Anal. 52 (2010) 300–304