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ORIGINAL ARTICLE

Celecoxib is an inhibitor of enzyme acetylcholinesterase

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Abstract

OBJECTIVES: Celecoxib is a nonsteroidal anti-inflammatory drug inhibiting enzyme cyclooxygenase-2 (COX-2). The drug was introduced in 1990s. In the work presented here, affinity of celecoxib to enzyme acetylcholinesterase (AChE) is inferred.
METHODS: Inhibition of human AChE by celecoxib was tested using standard spectrophotometric Ellman´s method and extrapolation of experimental data by Dixon plot. Interaction between AChE and celecoxib was also predicted by molecular docking using Swiss dock software.
RESULTS: A non-competitive mechanism of inhibition was revealed and equilibrium inhibitory constant equal to 313±40 μmol/l was determined. Comparing to AChE, celecoxib was not proved as an inhibitor of enzyme butyrylcholinesterase (BChE). The lowest ΔG was equal to –7.78 kcal/mol. In this case, celecoxib stacked sulfonamide moiety between TYR 337 and TYR 341 of alfa anionic subsite of active site. Cation-Π interactions appears to be responsible for the inhibition.
CONCLUSIONS: Though the here revealed and characterized inhibition has lower effect in real conditions than inhibition of COX-2, the inhibitory effect would be utilized in the next research and development of new AChE inhibitors.

INTRODUCTION

Celecoxib, a drug with proper chemical name 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl]benzenesulfonamide, belongs into group of nonsteroidal anti-inflammatory drugs (NSAID) like isobutylphenylpropanoic acid (ibuprofen) and acetylsalicylic acid. It was introduced as a selective inhibitor of COX-2 in mid 1990s (Penning *et al.* 1997; Riendeau *et al.* 1997). Structure of the drug is depicted in Figure 1.

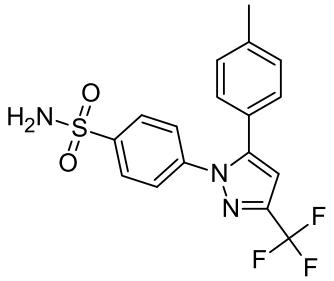

Fig. 1. Structure of celecoxib.

Comparing to older generation of NSAIDs, celecoxib does not inhibit COX-1 and it is fully selective to the COX-2. The selective inhibition was considered as the major advantage of the new drug (Geis 1999; Andrews *et al.* 1999). It is typically prescribed and used in cases of rheumatoid arthritis (Geis, Hubbard, Callison, *et al.* 1999), osteoarthritis (Geis, Hubbard, Woods, *et al.* 1999) and for acute pain management (Chen *et al.* 2015). There are also reports about celecoxib beneficial effects in therapy of cancers when it is given as a concurrent drug (Lin *et al.* 2006). Since the first use of celecoxib, there were also reports about side and adverse effects which confirm the idea that some of celecoxib pathways have remained untracked but physiological manifestations are perceptible. In an example, ability of celecoxib to improve manic episodes therapy was proved (Arabzadeh *et al.* 2015), celecoxib was proposed as a suitable agent for therapy of skin infections (Thangamani *et al.* 2015) and relation of celecoxib to acute pancreatitis (Hung *et al.* 2015) was also revealed.

Because of aromatic structural motives in celecoxib structure, it can be anticipated that the compound will have affinity toward enzyme AChE or even BChE. Especially reversible inhibitors of AChE have properties filling this condition because highly developed aromatic parts near active site of AChE, comparing to AChE, BChE has this part less developped (Pohanka 2012b). While AChE is an important part in the cholinergic nerves where it terminates neurotransmission by acetylcholine, BChE is a plasmatic enzyme with not known physiological function (Pohanka 2011, 2013a, 2015). Confirmation of the fact that celecoxib would be an inhibitor of enzyme AChE will help to understand its side pathways and will answer the question hot the pathways not directly associated with COX-2 can be impacted by celecoxib. In this paper, interaction between celecoxib and cholinergic system via inhibition of cholinesterases is hypothesized.

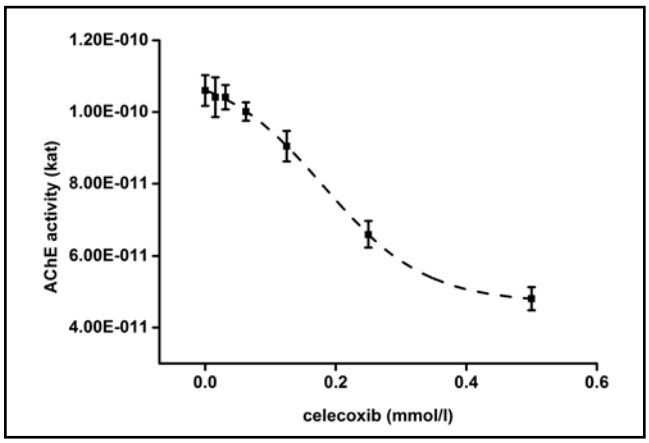

Fig. 2. Inhibition of AChE by celecoxib under fixed concentration of acetylthiocholine equal to 1 mmol/l. Error bars indicate standard deviations for n=5.

MATERIAL AND METHODS

Chemicals

Human recombinant AChE (specific activity ≥1,500 μmol/min/mg) and BChE (specific activity ≥500 μmol/min/mg) were bought from Sigma-Aldrich (Saint Louis, Missouri, USA) as lyophilized powders and used for assay purposes. Celecoxib was purchased from Cayman Chemical Company (Ann Arbor, MI, USA) as an analytical standard. Acetylthiocholine, butyrylthiocholine and 5,5´- dithiobis-(2-nitrobenzoic) acid (DTNB) were achieved from Litolab (Chudobin, Czech Republic). Phosphate buffered saline (PBS; pH 7.4) was bought from Sigma-Aldrich in a tablet form and prepared by dissolution in deionized water prepared by Aqua Osmotic 02 device (Aqua Osmotic; Tisnov; Czech Republic).

Enzymological test with celecoxib

Enzymological test was performed using modified Ellman´s method in which DTNB reacts with hydrolyzing product of acetylthiocholine (when AChE activity is assayed) or butyrylthiocholine (when BChE activity is assayed) (Pohanka 2012a, 2013c, 2013b). The used enzymes were diluted up to activity 1×10^{-9} kat per 100 μl of solution for 1 mmol/l substrate and standard ambient temperature and pressure (SATP) conditions. The assay was based on spectrophotometry with the standard PS disposable cuvettes. Following solutions were consequently added to the cuvette: 0.4 ml of DTNB 0.4 mg/ml in PBS, 100 μl of ethanol or celecoxib solved in ethanol, 100 μl of AChE or BChE solution in PBS and 300 μl of PBS. Finally, the reaction was started by addition of 100 μl of acetylthiocholine (AChE assay) or butyrylthiocholine (BChE assay). Absorbance was measured at 412 nm immediately after the mixture shaking and then after five minutes. Extinction coefficient ε=14,150 $l\times mol^{-1}\times cm^{-1}$ was used for calculation of enzyme activity in katals (Eyer *et al.* 2003).

Docking of celecoxib to AChE

Crystal structure of human AChE was taken from the cited paper as pdb file with molecule indication 4EY7 (Cheung *et al.* 2012). The molecule was processed and docked by Swiss dock software for reckoning of the lowest free energy of binding (Grosdidier *et al.* 2011b, 2011a). Chimera 1.10.2 software was chosen for data visualization in compliance with the reference (Pettersen *et al.* 2004).

Experimental data processing

Experimental data were processed in compliance with Dixon´s method (Dixon 1953; Cornish-Bowden 1973) as a reciprocal value of velocity against inhibitor concentration and inhibition constant K_i was calculated from the plots. Software Origin 9.1 (OriginLab Corporation, Northampton, MA, USA) was chosen for data plotting and constant calculation.

RESULTS AND DISCUSSION

Celecoxib was revealed to be an inhibitor of AChE in the given experiment. Inhibitory curve for a fixed concentration is depicted as Figure 2 and Dixon plot as Figure 3. Considering the Dixon plot, non-competitive mechanism of enzyme AChE was proved. Celecoxib has affinity toward the AChE in a sub-millimolar scale: equilibrium inhibitory constant was equal to 313±40 μmol/l. Because non-competitive inhibitory mechanism was revealed, numerical value of the equilibrium constant is equal to the median inhibitory concentration (IC_{50}) frequently used in pharmacology research (Cortes *et al.* 2001; Cer *et al.* 2009). Comparing to the results with AChE, celecoxib had significantly lower affinity toward BChE where equilibrium inhibitory constant equal to 2.35±0.22 mmol/l was achieved. It appears that celecoxib is not an inhibitor of the enzyme BChE because its equilibrium inhibitory constant does not reach sub-milimolar level which is considered as a threshold (Razinkov *et al.* 2002; Du *et al.* 2000).

Interaction between celecoxib and AChE was predicted by docking method as well. The lowest free energy of binding DG was equal to –7.78 kcal/mol for celecoxib bound into active site of AChE. In the lowest free energy, primary amine of sulfonamide moiety in celecoxib was stacked between TYR 337 and TYR 341 (Figure 4) probably by cation-Π interactions. PHE 295 and PHE 297 are in the vicinity of the sulfonamide moiety and they can also contribute to the cation-Π interactions as well. The amino acids are important part of alfa anionic subsite of active site in AChE (Pohanka 2011, 2015; Masson *et al.* 2002). It is inferred that this interaction is crucial for the here proved non-competitive inhibition. While the aromatic residues seem to be occupied by celecoxib in the active site, esteratic subsite stays probably intact. In a detailed look, SER 203 is a crucial amino acid in esteratic subsite of active site making hydrolysis of substrate acetylthiocholine (Rosenfeld, and Sultatos 2006; Kovarik *et al.* 2003). Despite relative proximity between SER 203 and methyl group of 4-methylphenyl moiety, there is no interaction causing blocking of the amino acid residuum. It is the reason why the inhibition is non-competitive and not competitive.

Considering relevance of the here presented findings, expected level of celecoxib in the body should be taken into account. Celecoxib is typically taken in doses 200 or 400 mg twice a day (Xiong *et al.* 2005; White *et al.* 2002). Expected peak concentration of celecoxib is 478 ng/ml within 3.8 hours (Krishnaiah *et al.* 2002). Calculating on molar scale, approximately 1.3 μmol/l level will be obtained. The level is too low to expect inhibition of AChE when celecoxib dosed in standard way and cholinergic manifestation of poisoning due to celecoxib can be expected only in cases of excessive overdosing. Ability to cross the blood brain barrier is a crucial factor predetermining whether the inhibitor of AChE is able to make impact on peripheral nerves only or whether inhibition of the enzyme in central nervous system is also presented. Regarding to the celecoxib, crossing through the barrier is possible and the drug can reach therapeutic concentration though the penetration rate is approximately one third of e.g. centrally acting diazepam (Novakova *et al.* 2014). Rate of the penetration can be further improved by closing the drug to liposomes (Ju *et al.* 2016) or microspheric particles (Vera *et al.* 2014).

Though typical plasma level of celecoxib is too low to cause significant inhibition of AChE, the inhibitory potency of celecoxib deserves attention because preparing of new classes of AChE inhibitors is an actual issue in pharmacology research and celecoxib would serve

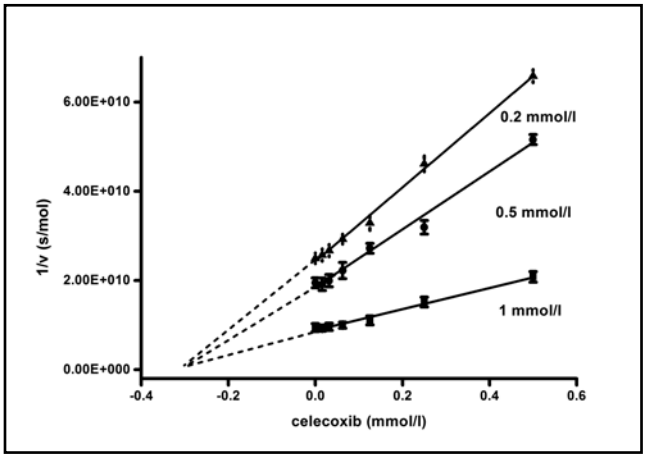

Fig. 3. Dixon plot for AChE inhibition by celecoxib. Final concentrations of acetylthiocholine in the solution are given beside each line. Error bars indicate standard deviations for n=5.

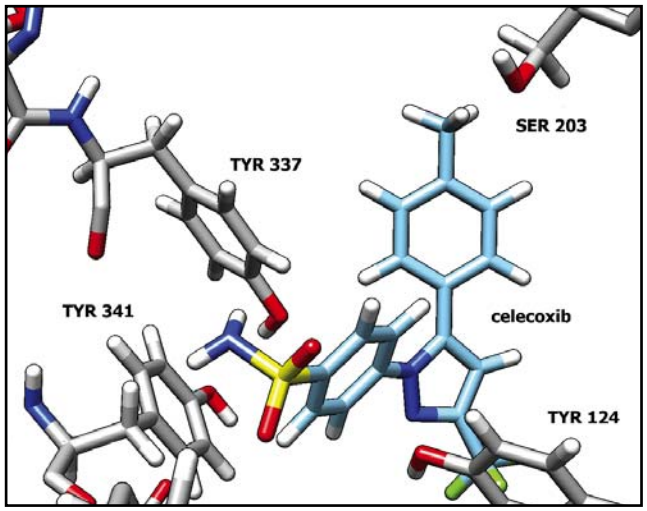

Fig. 4. Superposition of celecoxib in cavity of AChE active site. Molecule of celecoxib is highlighted by light blue. Atoms meaning: H – white, C – gray (in AChE) or light blue (in celecoxib), O – red, S – yellow, F –green, N – blue.

Tab. 1. Found facts about celecoxib interaction with AChE.

Parameter	Finding
Mechanism of inhibition	non-competitive
Equilibrium inhibitory constant (numerically equivalent to IC_{50} value)	313±40 μmol/l
Estimated DG	–7.78 kcal/mol (–32.6 kJ/mol)

as a lead structure in the research. Sulfonamide moiety is the significant structural motive which was responsible for the interaction with AChE described here. This structural motive on an aromatic moiety would be an interesting base for further development of drugs. Currently, selective inhibitors of AChE are extensively searched for application in autoimmune diseases, neurodegenerative disorders including Alzheimer disease and anti-inflammation (Pohanka 2012b, 2014b). Etiology of Alzheimer disease remains unclear but it appears that a neuroinflammation is associated with it (Pohanka 2014a). Potency of celecoxib to inhibit AChE would be interesting in this way because it would initiate the both protection from the inflammation and improve availability of the neurotransmitter acetylcholine. It should be emphasized that celecoxib is too weak inhibitor to make significant inhibition of AChE but it can act as an additive inhibitor to a standard drug. Basic facts about interaction between AChE and celecoxib are depicted in Table 1 for better lucidity in the issue.

CONCLUSIONS

Though the here revealed and characterized inhibition of AChE by celecoxib has lower effect in real conditions than inhibition of COX-2, the inhibitory effect mediated by celecoxib would be utilized in the next research and development of new AChE inhibitors. It is possible to prepare multi target drugs able to have anti-inflammatory effect by inhibition of COX-2 and by activation by so called cholinergic anti-inflammatory pathway via inhibition of AChE (Pohanka 2014b). Quantitative structure-activity relationship studies should be the next step in the research process.

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