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Slow-binding inhibition of acetylcholinesterase by an alkylammonium derivative of 6-methyluracil: Mechanism and possible advantages for myasthenia gravis treatment

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Abstract

© 2016 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society.Inhibition of human AChE (acetylcholinesterase) and BChE (butyrylcholinesterase) by an alkylammonium derivative of 6-methyluracil, C-547, a potential drug for the treatment of MG (myasthenia gravis) was studied. Kinetic analysis of AChE inhibition showed that C-547 is a slow-binding inhibitor of type B, i.e. after formation of the initial enzyme•inhibitor complex (Ki = 140 pM), an induced-fit step allows establishment of the final complex (Ki = 22 pM). The estimated koff is low, 0.05 -1 . On the other hand, reversible inhibition of human BChE is a fast-binding process of mixed-type (Ki = 1.77 μM; Ki = 3.17 μM). The crystal structure of mouse AChE complexed with C-547 was solved at 3.13 Å resolution. The complex is stabilized by cation-π , stacking and hydrogenbonding interactions. Molecular dynamics simulations of the binding/dissociation processes of C-547 and C-35 (a noncharged analogue) to mouse and human AChEs were performed. Molecular modelling on mouse and human AChE showed that the slow step results from an enzyme conformational change that allows C-547 to cross the bottleneck in the active-site gorge, followed by formation of tight complex, as observed in the crystal structure. In contrast, the related non-charged compound C-35 is not a slow-binding inhibitor. It does not cross the bottleneck because it is not sensitive to the electrostatic driving force to reach the bottom of the gorge. Thus C-547 is one of the most potent and selective reversible inhibitors of AChE with a long residence time, τ; = 20 min, longer than for other reversible inhibitors used in the treatment of MG. This makes C-547 a promising drug for the treatment of this disease.

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Keywords

6-methyluracil, Acetylcholinesterase, Butyrylcholinesterase, Molecular modelling, Slow-binding inhibition, X-ray structure.