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Characterization of a novel butyrylcholinesterase point mutation (p.Ala34Val), "silent" with mivacurium

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ABSTRACT

Butyrylcholinesterase deficiency is characterized by prolonged apnea after the use of muscle relaxants (suxamethonium or mivarcurium) in patients who have mutations in the *BCHE* gene. Here, we report a case of prolonged neuromuscular block after administration of mivacurium leading to the discovery of a novel *BCHE* variant (c.185C>T, p.Ala34Val). Inhibition studies, kinetic analysis and molecular dynamics were undertaken to understand how this mutation remote from the active center determines the "silent" phenotype. Low activity of patient plasma butyrylcholinesterase with butyrylthiocholine (BTC) and benzoylcholine, and values of dibucaine and fluoride numbers fit with a heterozygous enzyme of type atypical/silent. Kinetic analysis with succinyldithiocholine (SCdTC) as the substrate showed that Ala34Val BChE was inactive against this substrate. However, with BTC, the mutant enzyme was active, displaying an unexpected activation by excess substrate. Competitive inhibition of BTC by mivacurium gave a K_i = 1.35 mM consistent with the lack of activity with the related substrate SCdTC, and with the clinical data. Molecular dynamic simulations revealed the mechanism by which mutation Ala34Val determines the silent phenotype: a chain of intramolecular events leads to disruption of the catalytic triad, so that His438 no longer interacts with Ser198, but instead forms hydrogen bonds either with residues Glu197 and Trp82, or peripheral site residue Tyr332. However, at high BTC concentration, initial binding of substrate to the peripheral site triggers restoration of a functional catalytic triad, and activity with BTC.

1. Introduction

Butyrylcholinesterase (EC 3.1.1.8; BChE) also known as pseudocholinesterase and serum cholinesterase, is the sister enzyme of acetylcholinesterase (EC 3.1.1.7; AChE). It is present in most tissues including human plasma where its concentration is about 50 nM (4.2 mg per liter). Though BChE lacks obvious physiological functions, it is of toxicological and pharmacological importance in detoxifying or catabolising ester-containing drugs [1]. Furthermore, individuals deficient in BChE appear asymptomatic, apart from a heightened sensitivity to the muscle relaxants suxamethonium and mivacurium, two BChE carboxylester substrates used as myorelaxant [2]. In patients with usual BChE levels, these drugs are rapidly hydrolyzed in plasma and their duration of action is short (<10 min). BChE deficiency results in slower hydrolysis of these drugs and, consequently, a prolonged neuromuscular block, leading to apnea. Prolonged neuromuscular block occurs with BChE deficiencies of marked severity (impairment >70%). Although many acquired conditions may affect BChE activity (liver or renal diseases, malnutrition, pregnancy, malignancy), BChE deficiency is mainly due to mutations in the *BCHE* gene (MIM 177400) [2].

Prolonged apnea following injection of succinylcholine was first described in 1953 [3]. The genetics of BChE deficiency was described by Kalow and Genest in 1957 and is said to be a cornerstone in pharmacogenetics/pharmacogenomics [4]. The

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