87202_Auto_Edited.docx

WORD COUNT 3230
TIME SUBMITTED 11-OCT-2023 02:43PM
PAPER ID 103390201

Name of Journal: *World Journal of Experimental Medicine*
Manuscript NO: 87202
Manuscript Type: MINIREVIEWS

Emerging significance of butyrylcholinesterase

Gumpeny R Sridhar, Lakshmi Gumpeny

Abstract

Butyrylcholinesterase (BuChE; EC 3.1.1.8), an enzyme structurally related to acetylcholinesterase is widely distributed in the human body. It has a role in the detoxification of chemicals such as succinylcholine, which is a muscle relaxant used in anesthetic practice. It is well known because variant forms of the enzyme with little or no hydrolytic activity exist in some communities. Exposure to succinylcholine results in prolonged apnea. Its other functions include an ability to hydrolyze acetylcholine, the cholinergic neurotransmitter in the brain when its primary hydrolytic enzyme acetylcholinesterase is absent. To assess if it has other potential roles, it was studied in relation to insulin resistance, type 2 diabetes mellitus, cognition, hepatic disorders, cardiovascular and cerebrovascular disease, and inflammatory conditions. It appears to have an influenced and be influenced to these conditions *via* lipid metabolism. Individuals who lack enzyme activity are otherwise healthy other than having adverse outcomes on exposure to drugs that are hydrolyzed by it. Therefore, it is a candidate for the study of loss of function mutations in humans. Longitudinal studies in these individuals can assess if they are protective against metabolic diseases, or if the enzyme can be used as a biomarker for Alzheimer's disease and response to its drug treatment.

INTRODUCTION

Butyrylcholinesterase (BuChE), belongs to the esterase group of enzymes, which is a part of the serine hydrolase superfamily[1,2]. Esterases hydrolyze compounds that contain ester, amide and thioester bonds [1].. BuChE (EC 3.1.1.8) and acetylcholinesterase (AChE EC 3.1.1.7) share similar three-dimensional structure (3]. BuChe is believed to have resulted from a duplication of an ancestral acetylcholinesterase gene [4]. AChE is responsible for the hydrolysis of acetylcholine at the neuromuscular junction. The roles of BuChE are less well defined: it hydrolyses succinylcholine and bambuterol, used as muscle relaxants in anesthesiology [1]

INTRODUCTION

Butyrylcholinesterase (BuChE), belongs to the esterase group of enzymes, which is a part of the serine hydrolase superfamily[1,2]. Esterases hydrolyze compounds that contain ester, amide and thioester bonds [1].. BuChE (EC 3.1.1.8) and acetylcholinesterase (AChE EC 3.1.1.7) share similar three-dimensional structure (3]. BuChe is believed to have resulted from a duplication of an ancestral acetylcholinesterase gene [4]. AChE is responsible for the hydrolysis of acetylcholine at the neuromuscular junction. The roles of BuChE are less well defined: it hydrolyses succinylcholine and bambuterol, used as muscle relaxants in anesthesiology [1]

EVOLUTIONARY ASPECTS AND CHEMISTRY

BuChE gene (HGNC:983;MIM:177100) existed across life forms [5], including invertebrates [6].. The concentrations of BuChE exceed that of AChE in most tissues except the brain and muscle [3]. In the AChE knockout mouse model can compensate for the lack of AChE [7,8]. A convergent evolutionary mechanism is believed to have occurred between AChE and BuChE[6].

BuChE is mapped on chromosome 3 (3q26). It exists in plasma as four molecular forms. The tetrameric form comprises nearly 90% of total plasma cholinesterase activity[2] . In a monomer it consists of a common α/β hydrolase fold, flanked by α helices. The active site gorge volume of BuChE is larger than that of AChE[9], and is shaped like a bowl[2]. AChE though has nearly 40% more aromatic residues. The active gorge consists of an acylation site for catalysis and pockets for choline-bindin. It is rimmed by a peripheral

anionic site. The catalytic activity of BuChE depends on H-bond stabilization. In simulation studies inhibitors were shown to reach the catalytic cavity due to the flexible entrance of the gorge[10].

TISSUE DISTRIBUTION

BuChE is widely distributed in the lungs, plasma, brain and heart. Highest levels of BuChE mRNA are found in the liver, followed by the lung and the brain [4]. In the brain, it is present at neuromuscular junctions. BuChE is also expressed in the astrocytes of the brain. The close association of neurons and glia has been termed the 'tripartite synapse' whereby glia exchange information with neurons [11].

BIOLOGICAL ROLES OF BUTYRYLCHOLINESTERASE

The role of BuChE in anaesthetic practice is well recognized as a degrading enzyme of neuromuscular blockers succinylcholine and mivarurium [12]. Mutant forms have low or absent activity, resulting in prolonged apnea with the use of these muscle relaxants.

The levels are also lower in systemic diseases, liver disease, renal disease, malnutrition, malignancies and burns [13]. It participates in the first phase detoxification reaction against natural and exogenous toxins [14]. [Table 1]

In the brain, BuChE is present in the glial cells near the hippocampus and amygdala[15]. It may interact with ghrelin in the brain; in mice, increased expression of BChE is associated with low blood levels of ghrelin and reduced aggressive behaviour [16].

ASSOCIATION OF BUTYRYLCHOLINESTERASE AND ENVIRONMENTAL TOXINS

BuChE activity is principally found in 'tissues of first contact' such as lung, liver, skin and blood. It plays a role in activation of pro-drugs as well as metabolizing drugs to inactive forms. [17] . The biotransformation ability changes due to alterations in the macromolecular structure of the enzyme [17].

Other potential toxins that are inactivated by BuChE include carbamates and plants containing glucoalkaoids [18,19].

Reference range of AChE and BuChE were determined in 387 young and healthy individuals (201 men, 186 women aged between 18 and 45) [18]. This data can be used to assess altered levels of the enzyme in pathological states [20].

Gene polymorphisms of BuChE were studied in relation to groundwater fluoride toxicity [21]. In clinically healthy adults from Pakistan fluorosis was associated with elevated BuChE activity [21].

BUTYRYLCHOLINESTERASE, METABOLIC SYNDROME AND DIABETES MELLITUS

BuChE activity in rats was affected by dietary fat affected the BuChE activity in rats [22], perhaps due to increased release from hepatocytes [23]. Among subjects with type 2 diabetes from southern India, the plasma levels of BuChE were inversely related to serum cholesterol ($p<0.05$) [24]. The enzyme may not directly cause metabolic syndrome, but may serve as a marker [25]. Similar associations were reported between BuChE activity and weight in Mexican children [26]. Plasma BuChE were proposed as a marker of chronic low-grade inflammation [27]. In Japanese subjects (171 with type 2 diabetes and 88 controls) serum BuChE correlated with adiposity, serum lipids and HOMA-R [28]. To assess the risk of mortality with levels of BuChE, 813 subjects were followed up from 1985-1987 to 1996. Those in the lowest quintile of BuChE activity had higher mortality [29]. Alterations in body mass index were proposed to mediate changes in BuChE activity in healthy young men and women (age:18-25 years) [30].

Fruit extracts from Aronia melanocarpa affected BuChE activity [31]. Elevated BuChE levels predicted the development of type 2 diabetes [32] and of retinopathy [33]. Increased levels of BuChE may be associated with lower AChE levels, an anti-inflammatory molecule [34]. In cell culture studies, exposure to BuChE protects β cells of pancreas by reducing formation of toxic amylin oligomer formation [35].

BUTYRYLCHOLINESTERASE AND COGNITION

Alzheimer's disease (AD) that often accompanies aging is the most common cause of cognitive decline [36]. According to the cholinergic hypothesis, degeneration of cholinergic neurons in the basal forebrain results in cognitive dysfunction in Alzheimer's disease [37]. Others that act *via* the cholinergic system include amyloid deposition, tau phosphorylation, neuroinflammation and vascular damage (Pozzi *et al*, 2022) [38]. Acetylcholine, the neurochemical transmitter in the cholinergic synapses is

inactivated by AChE, and to a lesser extent by BuChE. The cholinergic system is part of the cholinergic anti-inflammatory pathway. The cholinergic hypothesis received additional support by cholinesterase inhibitor drugs which increase acetylcholinesterase at the synaptic cleft are effective in the management of AD [39].

Apart from its role in the cholinergic hypothesis, BuChE has been implicated in the deposition of amyloid. Amyloid hypothesis proposes that abnormal folding of β amyloid protein may contribute to the pathogenesis of Alzheimer's disease [40]. Variant form of the enzyme, called K-variant (Ala567Thr (A539T) may act synergistically with others such 4 allele of apolipoprotein E and iron as a risk factor for Alzheimer's disease [41,42].

BUTYRYLCHOLINESTERASE IN RELATION TO OTHER CONDITIONS

Liver Disorders

BuChE measurement is sometimes included in the panel of liver function tests due to its hepatic origin. It is an indicator of diseases such as acute hepatitis or cirrhosis of liver [14] . Before interpretation, one must exclude genetic variants and exposure to chemicals such as organophororus and carbamate insecticides.

BuChE levels are altered by inflammatory processes: they are reduced in acute inflammation and are normalized upon its resolution [43]. Lower levels were observed in malnourished children and in subjects with visceral undernutrition [44,45]. It can be used as a marker of nutritional status, especially among the elderly [46].

Acute coronary syndrome encompasses a range of conditions from angina pectoris to irreversible damage of myocardium. BuChE levels were used to differentiate healthy subjects from those with acute myocardial infarction (AMI). BuChE activity was lower in acute myocardial infarction (AMI) (n:85) compared with controls (n:45) (;<0.001) [47,48]. Similar observations were reported by Sulzgrubet *et al* (2015) BuChE levels were associated with mortality free survival in acute coronary syndrome. The strongest effect was observed among people aged 45-65 years [49]. Similar findings of mortality were reported in subjects undergoing veno-arterial extracorporal membrane oxygenation treatment after cardiac surgery [50].

Disorders of brain

In ischemic brain stroke, BuChE levels were measured in subjects with acute ischemic stroke (n:33; <12 h of onset) and 29 controls. BuChE activity was lower in acute stroke compared to controls [51].

BuChE levels were studied in 188 patients with traumatic brain injury within 72 h of injury. Non-survivors (n:42; 22.3%) had lower levels of BuChE activities [52]. The enzyme activity was acutely decreased in those who did not survive.

Pre-eclampsia

Pre-eclampsia, diagnosed in pregnancy, is characterized by hypertension, proteinuria and other maternal related dysfunction. BuChE levels were measured in 198 unrelated women having pre-eclampsia and 101 unrelated women with normal pregnancy. Pre-eclampsia was associated with lower BuChE activity [53].

Sepsis

Sepsis, presenting with acute organ dysfunction, often causes death in the intensive care setting. To identify the severity of sepsis, BuChE levels were used as a biomarker. Those who died within 90 days of admission had lower levels of BuChE. Admission levels of the enzyme could predict those who survived 90 days [54]. Measurement of BuChE could complement other ways of predicting the outcome of patients admitted in intensive care units. Using a newer definition of sepsis, 'life-threatening organ dysfunction due to a dysregulated host response to infection', Peng *et al* (2018) showed that lower levels of BuChE activity are an independent risk factor for 30-day death rate in sepsis-3 patients [55].

Infections

Six month outcome of subjects receiving highly active antiretroviral therapy for HIV infection, was assessed in relation to the levels of BuChE. Low levels of BuChE were seen in 25.5% (129/505) of subjects with infection,. In the first year, 16.6% patients died (n:84). Low BuChE levels were associated with a survival of 64.5+/-4.5% at one year compared to 87.6+/-1.8% in those with normal levels [56].

In Hansen's disease, genotyping of atypical BuChE allele (70G;rs1799807) and five additional SNPs reported a higher allele frequencies of 70G and genotypic 70DG rs1799807. It was suggested that atypical variants of the enzyme could predispose to the infection [57]. A possible role of interference with inflammatory response against the infective agent was proposed. Similarly, children with foot and mouth disease caused by enterovirus 71 infection had increased BuChE levels [58].

Fertility

The contribution of BuChE was assessed in relation to fertility. BuChE was measured in idiopathic unexplained infertility, a day before and a day after intrauterine insemination. A positive correlation between BuChE and total antioxidant activity on the day before the procedure. [59].

Other conditions

In preliminary studies, BuChE was measured in untreated Wilson's disease and in chronic obstructive pulmonary disease. BuChE was shown to be a biomarker in Wilson's disease; combined with measurement of serum ceruloplasmin, the diagnostic efficacy improved [60]. In subjects with chronic obstructive pulmonary disease (n:153), BuChE levels were elevated [61].

Studies in animals

Dogs with hypercortisolism, had elevated serum BuChE activity, related either to a direct effect of glucocorticoids or to changes in lipid metabolism associated with hypercortisolism [62]. Elevated salivary levels of BuChE were reported in dogs with parvovirus infections [63].

The underlying pathogenic mechanism in all these disparate conditions appears to involve dysregulation of the inflammatory response leading to adverse outcome.

Cholinergic control of inflammation

Inflammation is part of a physiological response that is protective against noxious environmental factors. In a recent review, Medzhitov (2021) proposed that inflammation ensures that homeostasis is retained and tissues retain their functional

and structural integrity [64]. It is regulated by chemicals from the immune system, hormones and by neural signals.

The vagus nerve conveys information from the brain to attenuate the inflammatory process. It regulates inflammation by integrating signals from the hypothalamic-pituitary-adrenal axis and through the cholinergic anti-inflammatory pathway [65]. AChE is a regulator of cholinergic nerve transmission by hydrolyzing the acetylcholinesterase. Bonaz *et al* (2016) proposed that the anti-inflammatory properties of vagus nerve may be used as therapy by stimulating the nerve [66].

BuChE may also influence the outcomes of Covid-19 infection *via* its effect on chronic low-grade inflammation, and may serve as a biomarker for its outcome.; subjects with variant BuChE may be studied in comparison to the native enzyme [34,64].

BChE activity was predictive of 28-day mortality in critically ill COVID-19 patients [67]. A recent report found low or declining levels of serum BuChE during hospitalization was associated with higher mortality[68].

KNOCK OUT ANIMAL MODELS TO ELUCIDATE FUNCTION OF GENES

In the field of functional genomics, gene-knock out models of animals can be employed to assess the function of genes. When a specific gene is artificially inactivated, the phenotypic effect gives information about the function of the inactivated gene [68] . Humans who harbor loss of function genes can provide better information than knock-out animal models [70]. The differences may relate to the existence of other regulators upstream or downstream of the gene and to environmental factors [71].

MacArthur (2012), reported that an average healthy person has 100 inactivated genes, of which 20 are homogenous [72]. Another study which used whole exome sequences from European populations (n:1432) reported that nearly 45% (n:76) of loss of function mutations were newly identified [[73]. Narasimhan *et al* (2016) studied the effects of rare gene knockouts in adults born of consanguineous marriage by performing exome sequencing in 3222 adults of Pakistani origin domiciled in Britain. Upon linking of sequence data to their lifelong health records, there was no relationship between those

with loss of function genes and their consultation for health issues or for prescription medication use. The latter were taken as surrogate markers for their state of health [74].

Loss of function mutations can result from: (a) nonsense SNP leading to a premature stop codon, producing a truncated protein sequence (b) splicing can be affected by a SNP at a canonical splice site (c) an insertion or deletion variants located in the gene coding region that can disrupt the full- length transcript leading to frameshifts (d) loss of function mutation can arise from a loss of an initiation codon [75].

Individuals with loss of function mutations who are apparently healthy were referred to as 'experiments of nature'. Studying them could help in the search for new drug targets and in identifying or exploring if such mutated genes could have beneficial effects [76].

Study of natural human knockouts and the correlation of genotype-phenotype is a field in its infancy which can provide insights into population genetics, and into the evolution of genes [69,71].

Butyrylcholinesterase and its variants qualify as natural human knockouts, because, other than prolonged apnea following exposure to succinylcholine, individuals harboring BuChE variants are apparently healthy [77].

SIGNIFICANCE OF STUDYING BUTYRYLCHOLINESTERASE VARIANTS

Unlike other gene knock-out animal models, variants of BuChE have a high prevalence in isolated ethnic groups, mainly south Indian from Vysya community, and certain Eskimos in western Alaska [34]. Li *et al* (2008) developed an animal BuChE gene knock-out model to test drug toxicity [78]. The model had a normal phenotype unless challenged with drug administration. The nicotinic receptor function was normal while muscarinic receptor function was altered, while. KO model showed altered cognitive functions. The effect of (R)-bambuterol, a specific and reversible inhibitor of BuChE suggested it may be used in the treatment of early cognitive decline [79].

BUTYRYLCHOLINESTERASE VARIANTS IN HUMANS

Lockridge *et al* (2016) reviewed the naturally occurring genetic variants of BuChE[80]. Thirty four loss of function mutations were identified; all of them were tolerated, viz, having a nonfunctional gene was compatible with life. Humans harboring silent BuchE

gene are healthy and fertile [77]. Lando *et al* (2003) reported that among healthy blood donors (n:2609), 59 had low plasma BuChE activity[80].

In the Netherlands Organisation for Applied Research (TNO) Prins Mauritis Laboratory and CDC, the frequency of BuChE mutations was nine out of 121,000 alleles. Some of the mutants showed complete absence of enzyme activity [81].

The commonest missent mutation is called the K-variant (Ala567Thr (AS39T), which is associated with 30% lower BuChE plasma activity compared to native BuChE. It results from an unknown mutation in a regulatory region [80]. Other variants are less common except in communities such as south Indian Vysyas or Eskimos. Where genotyping is not possible, estimating dibucaine and fluoride number could serve as a surrogate. Family studies in the south Indian state of Andhra Pradesh showed that various phenotypic forms of BuChE deficiency in inbred families (Data presented as oral presentation at 12th International Meeting on Cholinesterases-Sixth International Conference on Paraoxonases at Elche (Alicante, Spain) in 2015 (Supplement 1).

BuChe activity increases with progression of Alzheimer's disease and may eventually replace the function of AChE [82]. Individuals with BuCHE-K variant could have deleterious outcomes when donepezil is given to patients with mild cognitive impairment. It can therefore serve as a pharmacogenetic marker in the choice of drugs for cognitive impairment [82].

Reasons why non-functional proteins persisted were attributed to inbreeding, founder effect or their retention having no adverse effect. They could even confer evolutionary advantage, although the advantage if any, is currently unknown. Based on its ability to modulate lipid metabolism, it could perhaps lower the risk of cardiovascular disease despite high-fat diets [34].

Studies on human loss of function genes which were published did not identify variants of BuChE [72,73,74]. It is likely that specific endogamous ethnic groups where consanguineous marriages are prevalent and who have a high prevalence of variant BuChE enzyme were not sampled. To screen communities for genetic diseases where consanguinity if prevalent, advanced genetic testing using multigene panels and whole

exome sequencing are being considered [83,84]. Studies on variant BuChE do not require such complex technologies. When genomic testing is not possible, even phenotyping using fluoride and dibucaine number can be a first stage screening test[80].

CONCLUSION

Butyrylcholinesterase is an enzyme that plays a role in detoxification of noxious agents. The fact that it has been retained across evolution suggests it could play other physiological roles as well. Studies in a variety of conditions including metabolic disorders, Alzheimer's disease, coronary artery disease, cerebrovascular and infections and inflammatory states suggest that it regulates the inflammatory responses. Isolated populations harbor dysfunctional forms of the enzymes. They are healthy, until exposed to drugs or toxins such as succinylcholine when they have an aberrant response. These natural human knock-out models for butyrylcholinesterase can be followed up to understand their life trajectory and susceptibility or protection against diseases. [Table 2]

87202_Auto_Edited.docx

ORIGINALITY REPORT

2%
SIMILARITY INDEX

PRIMARY SOURCES

1	link.springer.com Internet	28 words — 1%
2	hal.archives-ouvertes.fr Internet	12 words — < 1%
3	www.mdpi.com Internet	12 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	OFF
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS