Research Article

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Investigation of anti-cholinesterase and anti-amyloidogenic activities of β-lactam antibiotics

β-laktam antibiyotiklerin antikolinesteraz ve antiamiloidojenik aktivitelerinin incelenmesi

https://doi.org/10.1515/tjb-2021-0277
Received December 3, 2021; accepted April 11, 2022;
published online May 23, 2022

Abstract

Objectives: Neuroinflammation is an important factor in the pathogenesis of neurodegenerative disesases. The following study aimed to clarify the effects of β-lactam antibiotics to the cholinergic system, on acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) activities, considering the structural differences of antibiotics, to evaluate the underlying mechanism of effects provided by protein-antibiotic interactions, and to clarify possible effects of the antibiotics on the aggregation of Aβ-peptides.
Methods: The inhibition/activation mechanisms for each antibiotic were examined kinetically by Ellman method. Destabilization effects of them on amyloid peptide fibrillation were examined and protein-ligand interactions were evaluated with most potent antibiotics by molecular docking studies.
Results: The most powerful inhibitions were detected by the inhibition studies of AChE with ceftazidime (CAZ) and BuChE with amoxicillin (AMX). CAZ was exhibited dose-related dual effect on AChE activity. CAZ was actually the dose-related modifier of AChE. At higher concentrations, CAZ was a nonessential activator of AChE. Molecular docking studies have been confirmed by kinetic studies. Interested β-lactam antibiotics did not prevent fibrillation rate as rifampicin.
Conclusions: Inhibition/activation behaviours of studied β-lactam antibiotics on both cholinesterases may suggest that cholinergic transmission is one of the crucially important components of the β-lactam antibiotics-induced central nervous system adverse reactions.

Keywords: β-lactam antibiotics; acetylcholinesterase; amyloid beta peptides; butyrylcholinesterase; molecular docking.

Öz

Giriş: Nöroinflamasyon, nörodejeneratif hastalıkların patogenezinde önemli bir faktördür. Bu çalışmada, β-laktam antibiyotiklerin yapısal özellikleri göz önüne alınarak kolinerjik sistem, asetilkolinesteraz (AChE), bütirilkolinesteraz (BuChE) aktivitelerine etkilerinin incelenmesi, protein-antibiyotik etkileşimleri kaynaklı mekanizmalarının açıklanması ve antibiyotiklerin Aβ-peptid agregasyonu üzerine olası etkilerinin araştırılması amaçlanmaktadır.
Gereç ve yöntem: Antibiyotikler için inhibisyon/aktivasyon mekanizmaları Ellman yöntemi kullanılarak kinetik olarak incelenmiştir. Amiloid peptit fibrilasyonu destabilize edici etkileri araştırılmış ve en etkin inhibitor özellik gösteren

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antibiyotikler için protein-ligand etkileşimleri moleküler modelleme yöntemleri ile gösterilmiştir.

Sonuçlar: Aktiviteler üzerine en güçlü inhibitör etkiler, AChE için seftazidim (CAZ) ve bütirilkolinestraz için ise amoksisilin (AMX) ile elde edilmiştir. CAZ, AChE aktivitesi üzerinde doz ilişkili dual etki göstermiş ve AChE için doz-ilişkili düzenleyici olarak değerlendirilmiştir. Yüksek derişimlerde CAZ AChE'ın aktivatörü gibi davranmaktadır. Moleküler modelleme çalışmaları kinetik sonuçlar ile doğrulanmıştır. Çalışılan β-laktam antibiyotikler amiloid fibrilasyonunu önlemede rifampisin kadar etkinlik göstermemiştir.

Tartışma: Çalışılan β-laktam antibiyotiklerin her iki kolinesteraz enzimi üzerindeki inhihbitör/aktivatör davranışları, kolinerjik iletimin β-laktam antibiyotik kaynaklı merkezi sinir sisteminde ortaya çıkabilen yan etkilerin önemli bileşenlerinden biri olduğunu düşündürmektedir.

Anahtar kelimeler: β-laktam antibiyotik; amiloid beta peptit; Asetilkolinesteraz; Bütirilkolinesteraz; moleküler modelleme.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by a progressive deterioration of the central nervous system (CNS), predominantly memory impairment and decline in cognitive functions. Whereas, the mechanism of the pathogenesis has not been clarified yet, cholinergic dysfunction, accumulation of β-amyloid peptides (Aβ-peptides) and tau protein hyperphosphorylation are accepted as main contributors of neurodegeneration. Also, oxidative stress and neuroinflammation play important roles in the development and progressive deterioration of AD [1–4].

Most of the current treatments for AD are based on the improvement of cholinergic system. Inhibition of acetylcholinesterase (AChE) activity provides the enhancement of the acetylcholine level in the brain of patients and reduces the symptoms of the disease. Acetylcholinesterase inhibitors (ACHIs) also have neuroprotective functions against aggregation of Aβ-peptides and oxidative stress [4, 5]. It was reported that the activity of butyrylcholinesterase (BChE) in the brain increases with age and is raised in AD. Recent studies have indicated that regulation of BChE activity has a crucial importance in late-stage AD patients, whose AChE is progressively lost [6, 7].

Neuroinflammation appears to have an important role in the progression of neurodegenerative diseases. Chronic inflammation alters the balance of pro- and anti-inflammatory signaling in the brain. The substances released within the CNS as a response against injury, infections or toxins can lead to inflammatory process and the immune response. Depending on the level of their activation, the immune responses can be beneficial or detrimental to the brain [2, 8].

β-Lactam antibiotics are known to be among the safest and most widely prescribed bactericidal agents that inhibit the synthesis of bacterial cell wall [9, 10]. Penicillins, cephalosporins, carbapenems and monobactams are the major groups of β-lactams (Figure 1). All β-lactam antibiotics contain a four membered β-lactam ring. Except monobactams, the β-lactam moiety is fused to 5- or 6-membered thiazolidine or dihydrothiazolidine ring [11–13].

Drug-induced CNS adverse reactions, neurotoxic and proconvulsant effects, of many β-lactam antibiotics have been reported [14–18]. Besides these, it has been demonstrated that some of the β-lactam antibiotics, including ampicillin, reveal a neuroprotective effect against ischemic damage in experimental models, *in vivo*, and also *in vitro* studies [19, 20]. Accumulation of antibiotics in the CNS can be excepted as a major determinant of neurotoxicity. However, mechanism(s) of it has not been fully clarified yet [14, 15, 19–21].

Possible contribution of β-lactam antibiotics to the cholinergic system, as a crucial contributer to adverse reactions, has not been investigated previously. Recently, it has been reported that some synthetic β-lactam derivatives reveal antioxidant activities and also have inhibitory effects on AChE activity [22]. The following study was performed (a) to clarify the effects of β-lactams on AChE and BChE activities, considering the structural differences of antibiotic, (b) to evaluate the underlying mechanism of effects provided by protein-antibiotic interactions, (c) to explain possible effects of the selected antibiotics on the aggregation of Aβ-peptides, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$. To our knowladge, this is the first comparative study to evaluate the effects of β-lactams on cholinergic transmission.

Materials and methods

Human recombinant AChE, equine serum BChE, Aβ-peptides, thioflavin (ThT) and β-lactam antibiotics: aztreonam (ATM), amoxicillin (AMX), ceftazidime (CAZ) and meropenem (MEM) were purchased from Sigma–Aldrich (MO, USA). All other chemicals and biochemicals were obtained from Sigma–Aldrich (MO, USA) or Merck (Darmstadt, Germany). All biochemical studies were performed in triplicate and data were expressed as mean ± SEM.

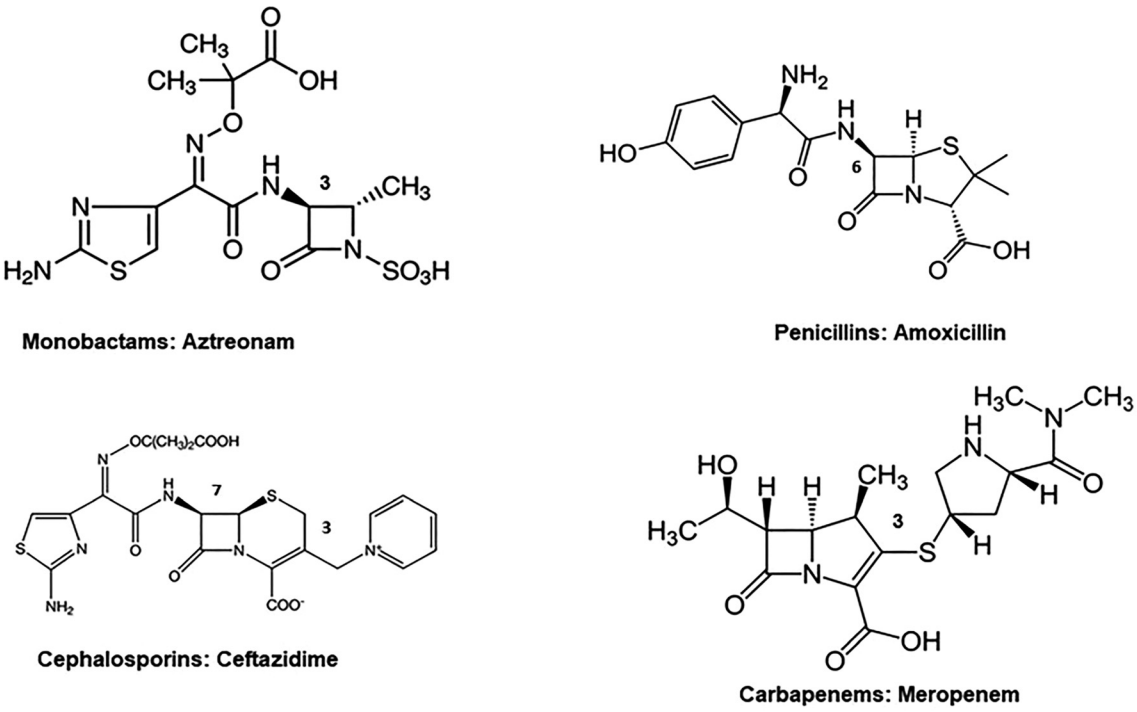


Figure 1: Structures of β-lactam antibiotics: major groups and selected member of each groups. Numbers represent the location of the side chains.

Activity determinations

AChE and BChE activities were carried out according to the modified Ellman method [23]. Activities were assayed at 25 °C, in 100 mM MOPS, pH 8.0, containing 0.05–0.5 mM acetylthiocholine iodide (ATC) or butyrylthiocholine iodide (BTC) as a substrate and 0.125 mM 5,5′-dithiobis (2-nitrobenzoic acid), DTNB. Reactions were initiated by the addition of enzyme, 0.125 U/mL for AChE and 0.5 U/mL for BChE, and monitored through the increase in absorbance at 412 nm against sample blank for a minute (Shimadzu-1601, Japan).

IC50 values were determined by the plots of activity vs. inhibitor concentrations and reported as mean ± SEM. Donepezil is used as the reference compound.

Reactions with β-lactam antibiotics

Antibiotic stock solutions in distilled water were prepared freshly. Activity studies were performed in the standard assay conditions in the range of 10–100 μmol/L antibiotic. In the absence of antibiotics, the enzyme was stable during the period of observations. Also, there was no reaction between antibiotics and DTNB [23].

Amyloid peptide fibrillation

The possible inhibitory effect of β-lactam antibiotics on the amyloid aggregation was tested by ThT fluorescence assay. ThT is a dye giving a characterictic fluorescence upon binding to peptides/polypeptides and proteins. The increase in the fluorescence intensity with respect to control is accepted as the increase of fibrillation/aggregation [24]. Commercially available peptides, dissolved in 100 mM potassium phosphate buffer pH 7.2, were incubated with/without 50 μmol/L of β-lactam antibiotics for 24 h. The flouresence intensities were obtained using 8 μM ThT and monitored by using spectrofluorimeter (Shimadzu RF-5301, Japan) at wavelengths 442 (exitation) and 482 (emmision). Rifampicin was also assayed as the standard for fibrillation destabilization.

Molecular modelling studies

Docking studies were performed using Molecular Operating Environment software, version 2018.0101 (MOE, Canada). The crystal structures of human AChE and BChE (PDB: 4EY7 [25] and 5NN0 [26]) were chosen as target proteins because of their resolution and co-crystallized ligands. All the non-bonded residues and water molecules were removed from the enzymes. The errors in the enzymes were corrected by the “Structure Preparation” application. The ligands were built using the MOE builder tool and energy was minimized using the Merck Molecular Force Field (MMFF94x, gradient: 0.05 kcal mol^{-1} $Å^{-1}$). Docking studies were performed using the Triangle Matcher method. The results were ranked with the London dG scoring function and rescored with the GBVI/WSA dG scoring function. The poses with the lowest S score were selected for the enzymes.

Results

The effect of β-lactam antibiotics on AChE activity

The possible inhibitory activities of antibiotics on cholinesterases were determined by IC_{50} values (Table 1). As seen in the Table 1, IC_{50} values were higher than the donepezil, 6.7 nM for AChE and 7.4 μmol/L for BChE [27].

Lineweaver–Burk (LB) plots including replots were constructed to evaluate the effects of studied β-lactam antibiotics on AChE activity. To verify the linearity and also to determine the values of enzyme-inhibitor dissociation constants (Ki), secondary plot(s) of LB and Dixon plots were used. LB replots indicated a linear inhibition of AChE by AMX and MEM. On the other hand, ATM inhibited AChE activity in a nonlinear hyperbolic manner. Besides these, CAZ revealed both inhibitory and activating effects on AChE activity in a dose–related manner. To clarify this dose-related dual effect of CAZ on AChE activity, inhibitory and activatory concentrations of CAZ were established by LB plot. The kinetic properties of each portion were examined separately. LB plots including replots and Dixon plots were constructed for each portion. To determine the enzyme-activator dissociation constant (Ka), and α and β values, again LB plot including secondary replot of 1/Δintercept vs. 1/CAZ were used.

Kinetic data were given in Table 2. As indicated in this table, Ki values were in the range of 39.1–120.1 μmol/L. Among all antibiotics, CAZ revealed the most powerful inhibitory activity on AChE. At lower concentrations, below 20 μmol/L, CAZ inhibited AChE activity in a linear noncompetitive manner with a Ki value of 39.1 ± 0.54 μmol/L (Figure 2A and B), whereas, above 20 μmol/L, CAZ was a nonessential activator of AChE, revealing nonlinear mixed-type activation with a Ka value of 200.7 ± 0.35 μmol/L (α=β=2.66 ± 0.011). In mechanistic point of view, the position of intersection of the LB plot was indicated the uncompetitive type activation, α=β>1. Also, Dixon plot was nonlinear, indicating the existence of multiple binding sites (Figure 2C–E) [28–30].

Table 1: IC_{50} values of antibiotics.

	IC_{50} (μM) ± SEM	
	AChE	BChE
Ceftazidime	37.2 ± 1.48[a]	55.8 ± 2.08
Amoxicillin	44.1 ± 1.14	65.1 ± 1.85
Meropenem	201.8 ± 4.65	312.4 ± 5.33
Aztreonam	70.7 ± 2.56	83.6 ± 1.77

Values are expressed as mean ± SEM. [a]50–100 μM inhibitory concentration.

The effect of β-lactam antibiotics on BChE activity

All studied β-lactam antibiotics inhibited BChE activity. The inhibition mechanisms for each antibiotic were clarified by using LB plots including their secondary plots and also Dixon plots. All of the inhibitions were nonlinear. The type of the inhibitions and related kinetic parameters were given in Table 2. As shown in this Table, Ki values were in the range of 31.3–145.2 μmol/L. All studied β-lactam antibiotics caused hyperbolic mixed-type inhibition (α = β < 1). Nonlinear Dixon plots confirmed partial inhibition. Among all β-lactams, AMX revealed the most powerful inhibitory activity on BChE, hyperbolic mixed-type inhibition with a Ki value of 31.3 ± 2.55 μmol/L (α=β= 0.41 ± 0.013) (Figure 3A–C). Under these conditions, a mixed-type inhibitor can produce the same effects of an uncompetitive inhibitor, called as hyperbolic or partial uncompetitive inhibition [31].

Table 2: The data of kinetic parameters of β-lactams on cholinesterases.

Antibiotic	Enzyme	Type of modification	Ki/Ka (μM)	α/β
Ceftazidime	AChE	Noncompetitive inhibition (0–20 μM)	39.1 ± 0.54	–
		Nonessential activation (50–100 μM)	200.7 ± 0.35	α=β=2.66 ± 0.011
	BChE	Hyperbolic mixed type inhibition	46.9 ± 0.88	α=β=0.33 ± 0.014
Amoxicillin	AChE	Uncompetitive inhibition	61.6 ± 0.15	–
	BChE	Hyperbolic mixed type inhibition	31.3 ± 2.55	α=β=0.41 ± 0.013
Aztreonam	AChE	Hyperbolic mixed type inhibition	113.9 ± 13.45	α=β=0.69 ± 0.016
	BChE	Hyperbolic mixed type inhibition	57.8 ± 9.4	α=β=0.38 ± 0.013
Meropenem	AChE	Uncompetitive inhibition	120.1 ± 2.88	–
	BChE	Hyperbolic mixed type inhibition	145.2 ± 17.25	α=β=0.53 ± 0.018

Values are expressed as mean ± SEM.

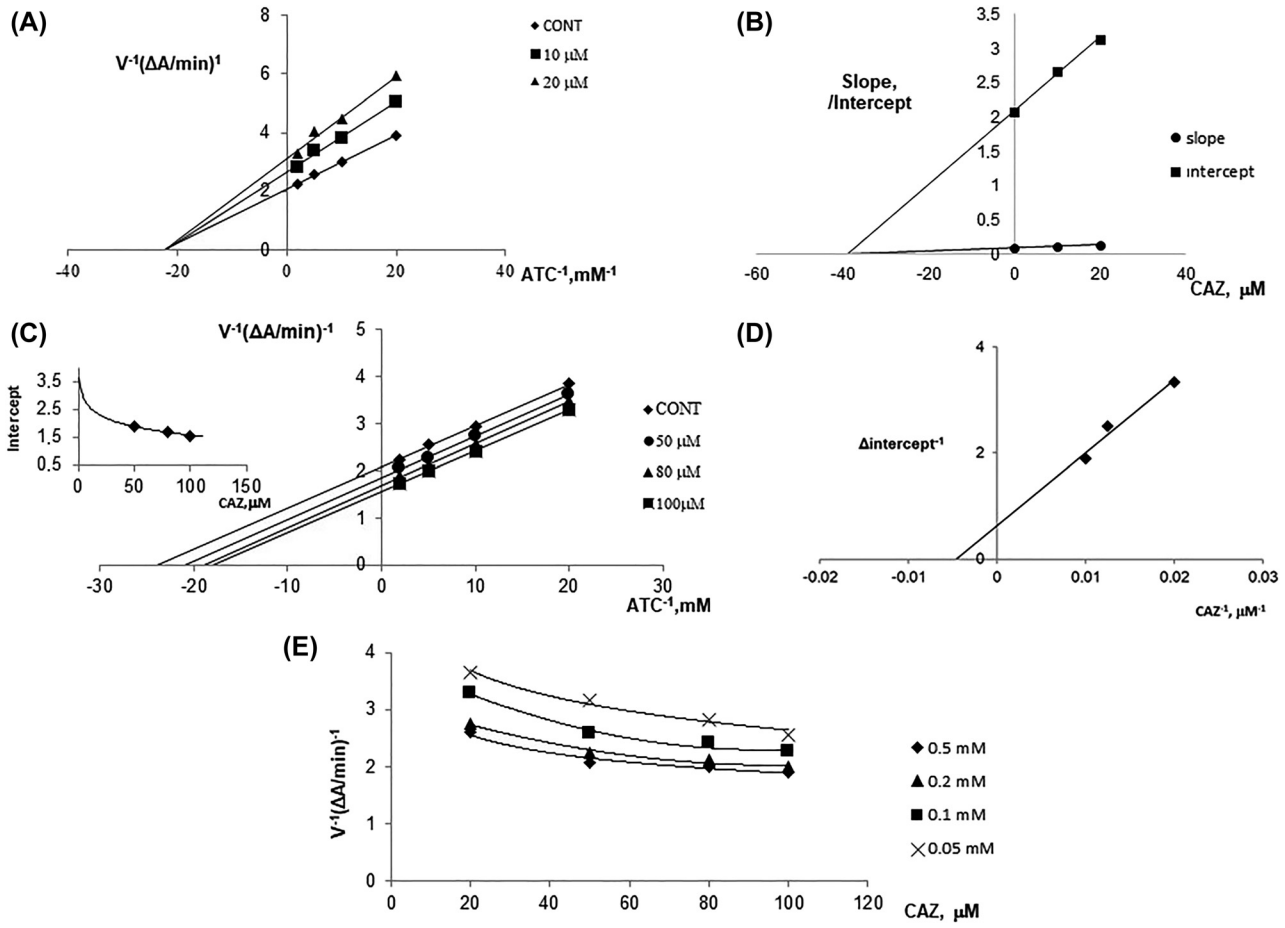


Figure 2: (A) The Lineweaver–Burk plot of AChE inhibition by CAZ, (B) secondary plot of Lineweaver–Burk plot i.e. v^{-1}-axis intercept/slope vs. [CAZ] replot (C) the Lineweaver–Burk plot of AChE activation by CAZ the insert: the plots of intercepts vs. CAZ (D) secondary plot of Lineweaver–Burk plot, $\Delta intercept^{-1}$/vs. [CAZ] replot (E) the Dixon plot.
Each point is the average of at least three determinations.

Destabilization effect of β-lactam antibiotics on amyloid peptide fibrillation

Destabilization effects of antibiotics on Aβ peptide fibriles were given in Figure 4. As shown in this figure, studied antibiotics revealed destabilizing effect only on $A\beta_{1-40}$–$A\beta_{1-40}$ fibriles aggregation. The percentage of inhibition of fibrillation was in the range of 2.6–36.5% compared to rifampicin.

Identification of protein-ligand interactions

Molecular docking studies were performed to explain the main interactions between interested β-lactams and cholinesterases. The β-lactam antibiotics having most potent inhibitory activity on cholinesterases were taken into consideration. These antibiotics were CAZ for AChE and AMX for BChE, as described in Table 2.

Interactions between AChE and CAZ

As seen in Figure 5, the active site cavity was completely occupied by CAZ. Docking studies revealed that at the central anionic site (CAS), there was a π- π interaction between indol residue of Trp86 and pyridinium group located at the C-3 position of a cephem skeleton. At the peripheral aromatic site (PAS), located at the mid gorge region, there was a σ- π interaction between indol residue of Trp286 and a methyl group located at carboxypropane part of the second side chain located at C-7 position of CAZ. At the active site gorge, there were four hydrogen bonds between AChE and CAZ. Two of them were formed between sulfur of aminothiazole group located at C-7 position of the second

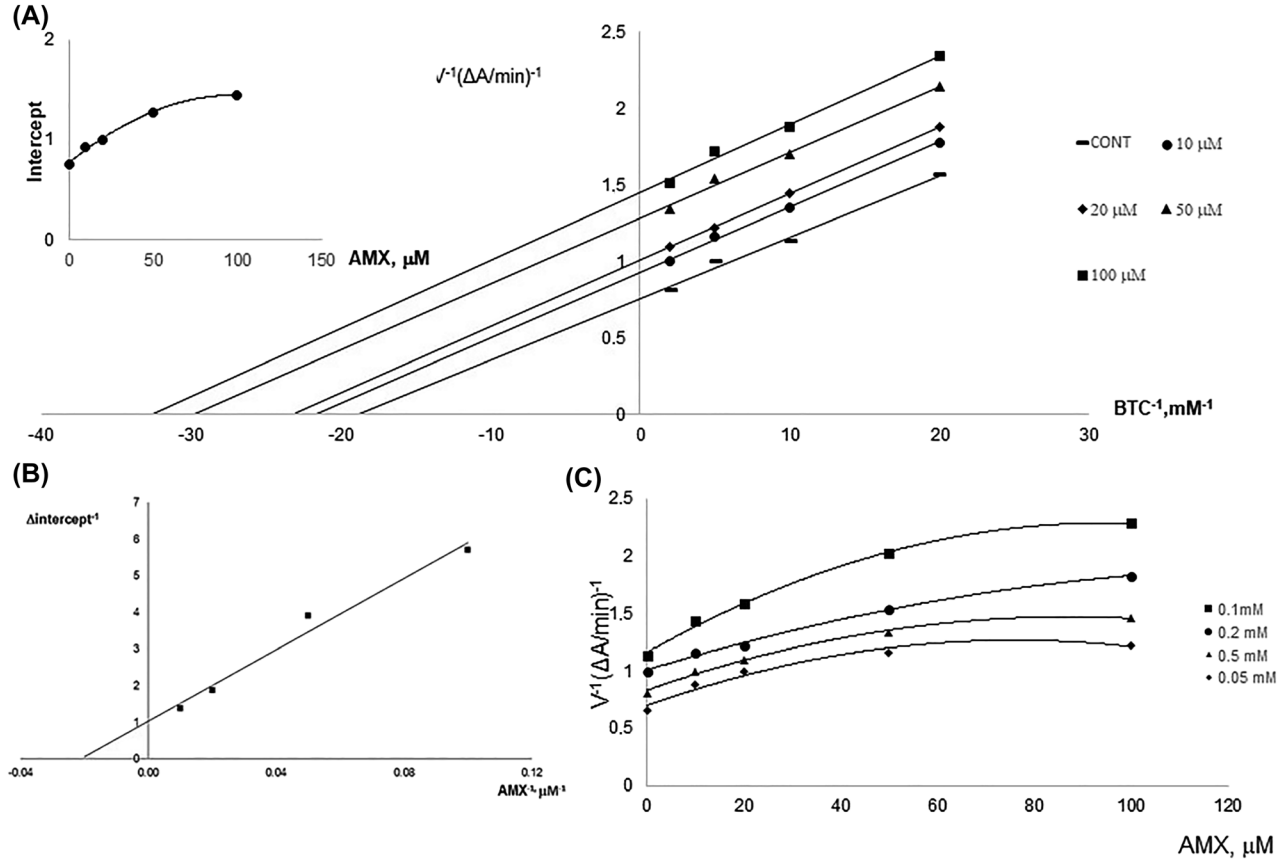


Figure 3: (A) The Lineweaver–Burk plot of BChE inhibition by AMX the insert: the plots of intercepts vs. AMX (B) secondary plot of Lineweaver–Burk plot, Δintercept^{-1} vs. [AMX] replot (C) the Dixon plot.
Each point is the average of at least three determinations.

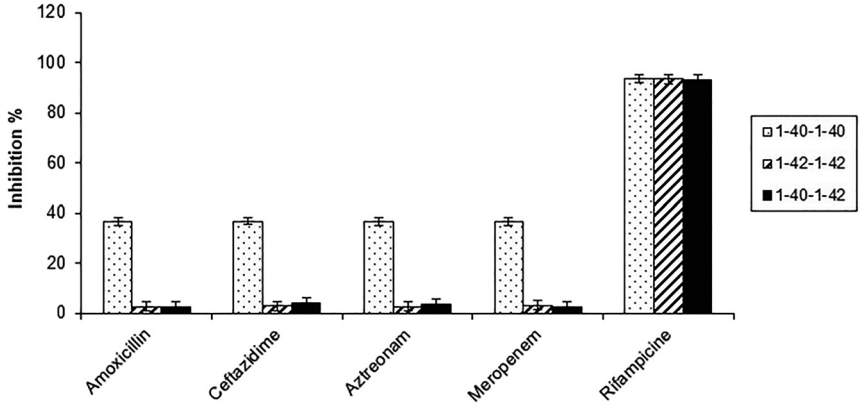


Figure 4: Inhibitory effect of studied β-lactams on amyloid peptide aggregation.

side chain of CAZ and Ser293 side chain and NH backbone of Arg296. The other hydrogen bond was again present at the second side chain of CAZ, between the carbonyl oxygen atom of oxyimino group and NH backbone of Phe295. On the other hand, the last hydrogen bond was formed between β-lactam, the carbonyl oxygen, and side chain of Tyr124 at the PAS. Hydrophobic residues of Phe297,Val294, Phe295,Leu76, Trp86, Phe338, Leu289 and Trp286 contributed to the protein-ligand interactions. Electrostatic interactions of polar residues of Ser293, Arg296, Asp74, Tyr124, Gly121, Gly120, Glu202, His447, Gly448, Ser203, Tyr341,Tyr337, Tyr72 were also participated in the protein-ligand interactions.

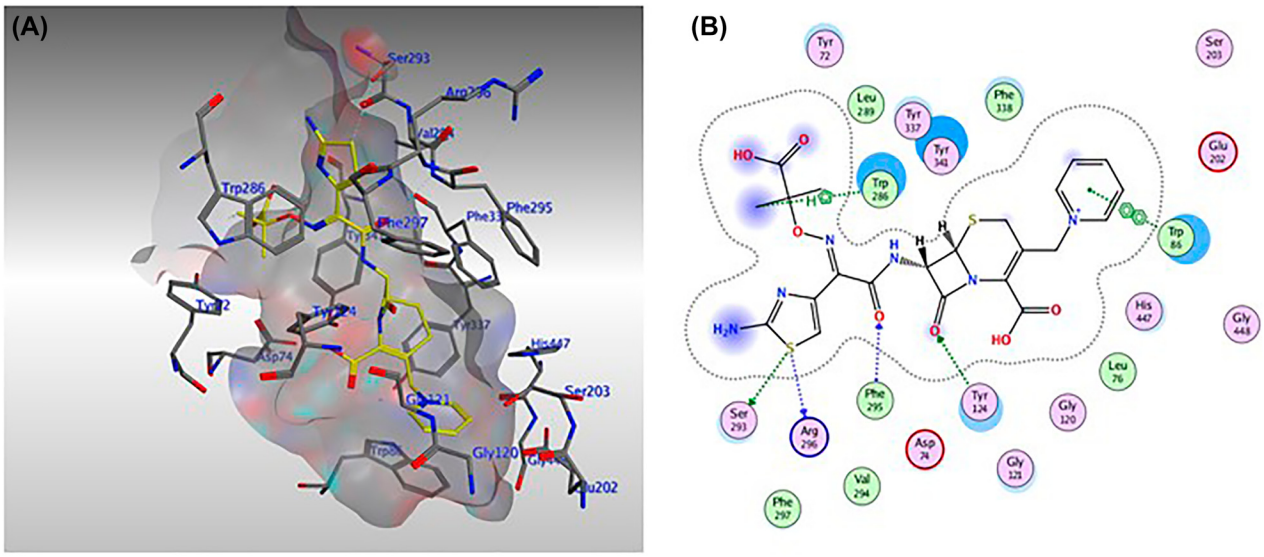


Figure 5: Docking interactions of CAZ on AChE.
(A) 3D (B) 2D representation.

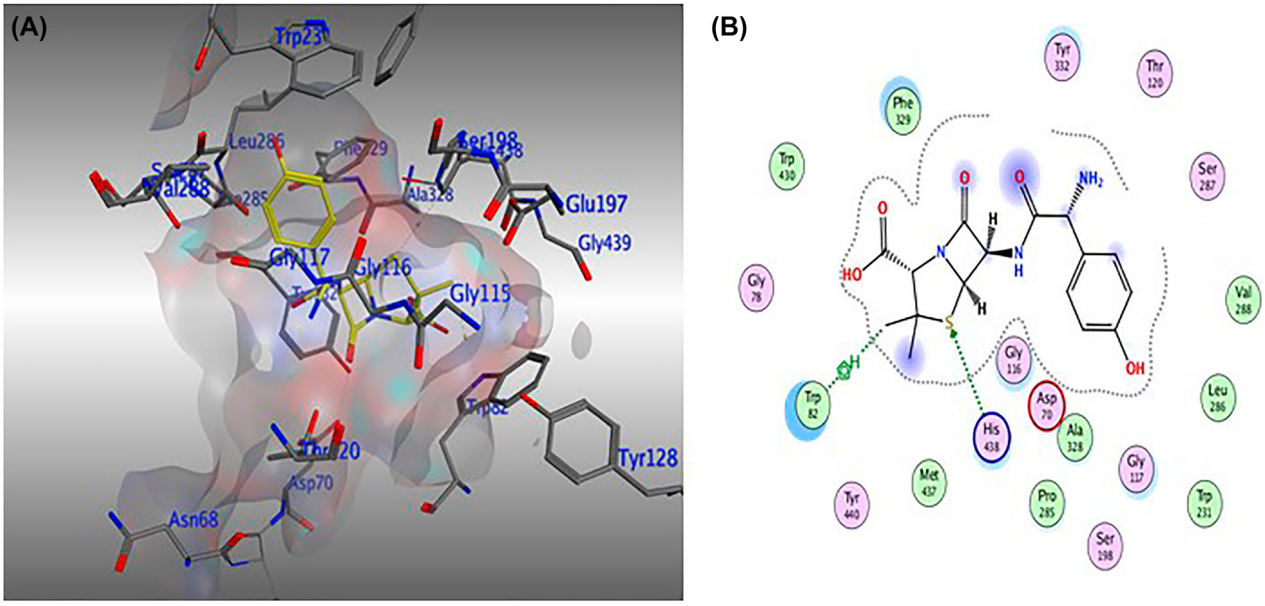


Figure 6: Docking interactions of AMX on BChE.
(A) 3D (B) 2D representation.

Interactions between BChE and AMX

Protein-ligand interactions between BChE and AMX were given in Figure 6. The active site cavity of BChE is larger than that of AChE and catalytic triad is located at the bottom of the gorge. As seen in figure, the cavity was almost occupied by AMX. At catalytic anionic site (CAS), there was a σ–π interaction between indol residue of Trp82 and one of the methyl group located at C-3 position of the thiazolidine ring. Docking studies revealed the existence of one hydrogen bond between sulfur of the thiazolidine ring and imidazole side chain of His438, one of the member of catalytic triad. The hydrophobic residues of Phe329, Trp430, Trp82, Met437, Ala328, Pro285, Trp231, Leu286 and, Val288 contributed to the protein-ligand interaction. Besides these, electrostatic interactions of polar residues of Tyr332, Thr120, Ser287, Gly117,

Gly116, Ser198, Asp70, Tyr440 and Gly78 residues were also contributed to the protein-ligand interactions.

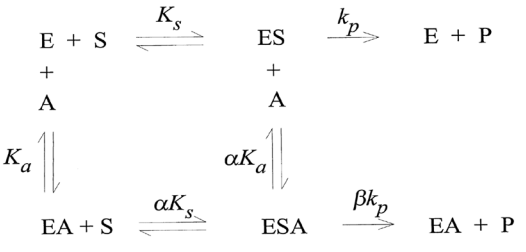
Discussion

In the present study, all studied β-lactam antibiotics inhibited the AChE and BChE activities reversibly. All the inhibitions were nonlinear except inhibition of AChE by CAZ, AMX and MEM. The most powerful inhibitions, with the lowest Ki values were detected for the inhibition of AChE with CAZ and BChE with AMX. CAZ was exhibited dose-related dual effect on AChE activity behaving as the dose-related modifier of AChE. Its dual effect as inhibitor/activator depended on the modifier level, but not to the substrate concentration.

At lower concentrations than 20 μmol/L, CAZ inhibited AChE activity in a linear noncompetitive manner (Figure 2A and B). Data have indicated that CAZ has a Ki value of 39.1 μmol/L which is lower than Ks value, e.i. 45.45 μmol/L meaning that the affinity of CAZ to AChE was higher than its substrate. At indicated concentrations of CAZ, decreased V_{max} value was related to the formation of nonproductive ESI complex. On the other hand, CAZ changed its apparent effect on AChE at higher concentrations above 20 μmol/L. This effect was a nonessential activation with Ka value of 200.7 μmol/L (α=β=2.66).

The kinetic model of nonessential activation is similar to a general model for nonlinear hyperbolic inhibition except there is an activation instead of inhibition. Kinetic scheme of the nonessential activation was given in Scheme 1 [28].

In the present study, data have revealed that CAZ modulates the kinetic parameters by decreasing the affinity of binding of the substrate to the enzyme (α=2.66) and increasing the catalytic rate constant (k_p) value by β, i.e. 2.66 (Figure 2C–E). The high value of α reflects the decreased affinity of substrate/activator to the EA/ES complex respectively. V_{max} value was enhanced in the presence of high



Scheme 1: The nonessential activation model. Ks, S, E, A, Ka and kp are the dissociation constant of the substrate from the enzyme, substrate, enzyme, activator, activator dissociation constant and catalytic rate constant respectively.

concentrations of CAZ. As seen in Scheme 1, the resulting ESA complex was contributed product formation by a factor of 2.66 time's catalytic rate constant (k_p).

In a report investigating the effects of benzalkonium on cholinesterases, similar results (inhibition/activation effects of effectors) were obtained depending on the charge and the concentration of substrates [29]. However, in our model the dual action of CAZ as a modifier, was not substrate concentration dependent.

Our molecular docking studies have been confirmed by kinetic studies. The most powerful antibiotics, CAZ for AChE and AMX for BChE, interacted with some amino acid residues belonging to PAS, CAS and acyl binding site of active site gorges. Besides existence of H-bonds between CAZ and amino acid residues of AChE at the active site cavity (Figure 5), π–π interaction between indol group of Trp86 and pyridinium group of CAZ and σ–π interaction between Trp286 and carboxypropane group of CAZ were critically important. As reported previously, Trp286 and Trp86 residues are located at the PAS and CAS of AChE active site gorge, respectively. Interaction of these residues causes distinct conformations of the active site to occur and changes the functionality of the enzyme. At high concentration of CAZ, modulation of the conformational change of active site is probably responsible from the nonessential activation of AChE. In other words, CAZ, at high concentrations, was stabilized the active site gorge as activating conformation for catalysis [8, 32]. β-lactam antibiotics having a direct interaction with the catalytic triad residues revealed the most potent inhibitory activity, as in the case of the inhibiton studies of BChE. Besides σ–π interaction between Trp82 and the methyl group of the thiazolidine ring of AMX, there was a direct interaction with one of the residues of catalytic triad, i.e. His438 (Figure 6).

In our study, the effect of diversity of molecular structures of studied β-lactam antibiotics on their cholinesterase inhibitory potentials have examined. As seen in Figure 1, major structural difference(s) are related to their core ring structures. The structure of ATM is different than other β-lactams. It has monocyclic beta-lactam ring. The second difference is related to the nature and the location at the C-skeleton of side chains. In AMX, the most potent BChE inhibitor, there is a thiazolidine ring and side chain attached to the secondary amine of penicillin skeleton, at the C-6 position. On the other hand, CAZ has a dihydrothiazine ring and two side chains at the C-3 and C-7 positions of cephem skeleton. The last difference of the most potent inhibitors of both cholinesterases is related to their ionizable functional groups at physiological pH. Considering these major differences, one can suggest that β-lactam ring fused with either dihydrothiazine or thiazolidine with side chains

located at the C-6 or C-7 position and negative net charge (i.e. –1) are the essential factors for the determination of degree of inhibition patterns of cholinestereases.

All β-lactam antibiotics, hydrophilic compounds, can cross the blood brain barrier and enter cerebrospinal fluid (CSF) through paracellular pathways. Their transport depends on the opening of the tight junctions and an active system (has a low affinity and capacity) that transports antibiotics from blood to CSF [33]. It has been reported that, under normal circumstances, the CSF concentration of β-lactams is low owing to limited transport across the blood-brain barrier [17]. Evidences have indicated that alterations of the blood-brain barrier (e.g. aging, renal failure/insufficiency, some CNS related diseases including AD) cause the elevation/enhancement of the concentration of the antibiotics in the CSF [4, 16, 34]. Accumulation of the compound in the CNS (particularly in excessive dosage) and impaired renal clearance are the most important factors to determine the antibiotic-associated neurotoxicity [14, 35]. It has been reported that neurotoxic effects related to β-lactam compounds involve the interference/inhibition of gamma-aminobutryric acid (GABA) binding to GABAA receptors. Evidences have indicated that not all β-lactam antibiotics act in the same manner. Penicillin inhibits GABAA receptors in a noncompetitive and voltage-dependent manner. On the other hand, cephalosporins reveal antagonistic action at the GABAA receptor complex [35]. The reduction in GABA-mediated inhibition on the inward chloride current permits excitatory cortical afferents to produce CNS excitation or trigger epileptiform discharges [36, 37]. Accumulation of both β-lactam antibiotics and toxic organic acids in CSF and in the brain tissue may directly or indirectly contribute to the induction of adverse reactions, including epileptogenic activity [17].

Within β-lactam antibiotics, cephalosporins appear to be an important cause of CNS adverse drug reactions. Lacroix et al. [15] have reported the cephalosporin plasma levels for patients with CNS adverse drug reactions with a median plasma level of CAZ as 104 mg/L that is higher than plasma levels of standards (40–80 mg/L). In the study conducted by Fong et al. [38], mean CAZ concentration in CSF of patients without inflamed meninges was given as 0.8 μg/mL after i.v. infusion of 2–3 g of CAZ. On the other hand, mean CAZ concentration in CSF of patients with meningitis was reported as 22.6 μg/mL. To clarify the effect of CAZ on AChE activity, reported plasma and CSF concentrations were converted to the μmol/L. The elevation of CSF mean CAZ concentration 41.32 μM (22.60 mg/L), indicated the increased permeability of the brain-blood barrier in patients with meningitis with respect to given values [38]. The high level of CAZ in CSF in patients with meningitis comparing to the CSF mean CAZ value of patients without meningitis may be expected as an indication of modulation of cholinergic system by CAZ. Since, CAZ behaves as a nonessential activator of AChE and a hyperbolic mixed-type inhibitor of BChE (Table 2). The median CAZ plasma level in patients with CNS adverse drug reactions was found as 190.13 μmol/L after conversion of reported value, i.e. 104 mg/L [15]. At this concentration, CAZ activates AChE. In other words, cholinergic transmission can be expected as one of the crucial trigger for the adverse reactions of CAZ, directly or indirectly.

It is mainly reported that there is an association between bacterial/viral infections and AD progression and many experiments were carried on to identify/clarify the therapeutic potential of antimicrobial and antiviral drugs considering their mechanisms of action and their impact on Aβ-peptide levels [2]. $A\beta_{1-42}$ is the major insoluble form present in the patients with AD. The oligomeric and insoluble forms of Aβ are toxic to brain cells, directly contributing to the pathogenesis of AD [1]. It has been suggested that Aβ-peptide is involved also in the protection and repair of the CNS and regulates synaptic function and contributes to memory consolidation [39]. Iqbal et al. [2] have reported that Aβ peptides play a beneficial role in immunity, that is why the aim of treatment should not be to eradicate the compound completely. In the present study, there was no significant alterations of the inhibitory effects of interested β-lactam antibiotics on the aggregation of fibriles, whereas the aggregation of $A\beta_{1-40}$ fibriles were sensitive to the treatment with studied β-lactam antibiotics. One can be proposed that the possible effect of the interested antibiotics on the fibrillation process depends on the content of Aβ isoforms.

Our data have indicated that all interested β-lactam antibiotics behave just like ChEIs. Besides inhibition of ChEs activities in various degrees, CAZ also acts as a nonessential activator of AChE in a dose-related manner. Inhibition/activation behaviours of studied β-lactam antibiotics on both cholinesterases may suggest that cholinergic transmission is one of the crucially important components of the β-lactam antibiotics-induced CNS adverse reactions.

Research funding: This study was funded by Başkent University after the approval of Medical Research Committee (Project no: DA21/15). Each author equally contributed to this study.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest: Authors state no conflict of interest.
Informed consent: Not applicable.
Ethical approval: Not applicable.

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