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Monoclonal antibodies to human butyrylcholinesterase reactive with butyrylcholinesterase in animal plasma

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ABSTRACT

Five mouse anti-human butyrylcholinesterase (BChE) monoclonal antibodies bind tightly to native human BChE with nanomolar dissociation constants. Pairing analysis in the Octet system identified the monoclonal antibodies that bind to overlapping and independent epitopes on human BChE. The nucleotide and amino acid sequences of 4 monoclonal antibodies are deposited in GenBank. Our goal was to determine which of the 5 monoclonal antibodies recognize BChE in the plasma of animals. Binding of monoclonal antibodies 11D8, B2 18–5, B2 12–1, mAb2 and 3E8 to BChE in animal plasma was measured using antibody immobilized on Pansorbin cells and on Dynabeads Protein G. A third method visualized binding by the shift of BChE activity bands on nondenaturing gels stained for BChE activity. Gels were counterstained for carboxylesterase activity. The three methods agreed that B2 18–5 and mAb2 have broad species specificity, but the other monoclonal antibodies interacted only with human BChE, the exception being 3E8, which also bound chicken BChE. B2 18–5 and mAb2 recognized BChE in human, rhesus monkey, horse, cat, and tiger plasma. A weak response was found with rabbit BChE. Monoclonal mAb2, but not B2 18–5, bound pig and bovine BChE. Gels stained for carboxylesterase activity confirmed that plasma from humans, monkey, pig, chicken, and cow does not contain carboxylesterase, but plasma from horse, cat, tiger, rabbit, guinea pig, mouse, and rat has carboxylesterase. Rabbit plasma carboxylesterase hydrolyzes butyrylthiocholine. In conclusion monoclonal antibodies B2 18–5 and mAb2 can be used to immuno extract BChE from the plasma of humans, monkey and other animals.

1. Introduction

Butyrylcholinesterase (BChE; EC 3.1.1.8) is a serine esterase that catalyzes the hydrolysis of acetylcholine, butyrylcholine, benzoylcholine, indoxyl acetate, p-nitrophenyl acetate, phenyl acetate, alpha-naphthyl acetate, aspirin, procaine, succinyldicholine, mivacurium, cocaine, heroin, irinotecan, bambuterol and octanoyl ghrelin [1–4]. Some of these esters are used as drugs to treat various human conditions. BChE also serves as a stoichiometric bioscavenger for organophosphorus pesticides and nerve agents, thus protecting acetylcholinesterase from irreversible inhibition by these toxic compounds [5].

Many drugs are synthesized as esters to neutralize their charge, thus allowing the drug to be absorbed through the gut and through cell membranes including cells in the brain. The esterified drug may be a prodrug that requires hydrolysis of the ester bond to become the active drug. For example, the antiasthma prodrug bambuterol is hydrolyzed to the active drug terbutaline by BChE [6]. Other ester drugs are detoxified by BChE. For example, BChE hydrolyzes cocaine to the pharmacologically inactive metabolites, ecgonine methyl ester and benzoic acid [7–9]. New drugs are tested in

Abbreviations: HuBChE, human butyrylcholinesterase; BSA, bovine serum albumin; CES, carboxylesterase; U, units of activity.

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animals before they are tested in humans. Standard laboratory animals including the mouse, rat, guinea pig, and monkey have levels of BChE in plasma that are not representative of the level of BChE in human plasma. The rat has 30-fold less plasma BChE compared to humans. Rhesus monkey has 2–3 fold higher levels of BChE in plasma than humans. Another difference between animals and humans is that mouse, rat, and guinea pig have carboxylesterase in plasma, whereas humans and monkeys have none [4]. The plasma carboxylesterase in animals degrades drug esters rapidly, giving an inaccurate pharmacokinetic profile for drug metabolism in humans. The plasma carboxylesterase knockout mouse, ES1−/− (Jackson Laboratory stock number 014096), can be used as a model for how humans metabolize an ester drug [10,11].

Knowledge of the identity of the esterase involved in metabolism of a drug is important because it leads to an understanding of individual differences in response to drugs. The atypical and silent variants of human BChE do not hydrolyze succinylcholine and mivacurium; consequently people with these genetic variants are unable to breathe for up to 2 h from a dose intended to paralyze for 3 min [12]. More than 70 variants of human BChE have been recognized [13]. Animal studies that aim to identify the role of BChE in drug metabolism may benefit by using an immobilized antibody to capture BChE. The captured BChE has activity and can be used directly to measure the role of BChE in ester hydrolysis, as we have done for octanoyl ghrelin [14]. Mrvova et al. produced two monoclonal antibodies in BChE knockout mice that recognize BChE in mouse, rat and dog plasma [15].

In a previous report [16] we characterized 5 mouse anti-human BChE monoclonal antibodies. We reported similar nanomolar binding affinities for all 5 monoclonal antibodies, but variable performance in their ability to immunopurify BChE from human plasma. Octet pairing analysis was carried out by immobilizing pure human BChE on a chip followed by measurement of the change in the interference pattern when antibody flowed over the chip. Pairing analysis showed that two pairs of antibodies, mAb2 and B2 12–1, as well as mAb2 and 3E8, bound to independent epitopes on human BChE and were therefore useful in sandwich assays. We deposited the nucleotide and amino acid sequences of 4 monoclonal antibodies in GenBank. The goal of the present work was to identify the monoclonal antibodies that recognize BChE in the plasma of animals. We describe 5 monoclonal antibodies to human BChE, two of which have broad specificity enabling them to recognize BChE in the blood of human, monkey, horse, cat, and tiger.

2. Materials and methods

2.1. Reagents

2.1.1. Pansorbin cells 1 g (Calbiochem #507862) coated with 1 ml of rabbit anti-mouse IgG H + L (Jackson ImmunoResearch #315-001-003)

Dynabeads Protein G (Life Technologies #10004D). ImmunoPure immobilized Protein G (Pierce #20398). Human plasma Na Citrate pooled from 10 donors (UNMC blood bank); rhesus monkey plasma Na Heparin and horse serum (Atlanta Biologicals); cat plasma Na Citrate (Pel-Freeze); Bengal Tiger plasma EDTA anticoagulant (Omaha Henry Doorly Zoo); New Zealand white rabbit plasma Na Heparin, porcine serum, and rat serum (RJO Biologicals); guinea pig plasma Na Heparin and mouse strain 129 serum (Taconic); chicken plasma Na Heparin (Innovative Research Inc.); bovine calf serum (General Scientific Lab). Acetylthiocholine iodide, butyrylthiocholine iodide and ethopropazine hydrochloride (Sigma–Aldrich).

2.1.2. Monoclonal antibodies to HuBChE

Mouse anti-human BChE monoclonal antibodies were from the following sources. Monoclonal 3E8 was purchased from Thermo Scientific Pierce (HAH 002-01-02); the original 3E8 monoclonal was made in Denmark [17]. Hybridoma cell line 11D8 was a gift from Dr. Eric Krejci and Dr. Anna Hrabovska [18]. We purified the 11D8 monoclonal from culture medium on Protein G-Sepharose. Hybridoma cell lines B2 18–5 and B2 12–1 were a gift from Dr. Stephen Brimijoin [19]. The cells, having been stored at −80 °C for 30 years, failed to grow but their RNA was still intact. Syd Labs Inc (Natick, MA) made the B2 18–5 and B2 12–1 monoclonal antibodies by recombinant DNA methods using mRNA as a template to make cDNA, followed by ligation of the cDNA into vectors that contained the constant regions, coexpression of the light and heavy chains in Chinese Hamster Ovary cells, and purification on protein A-Sepharose. Ascites fluid from B2 18–5 and B2 12–1 was available for comparison to the recombinant antibodies. The mAb2 monoclonal was made in France [20]. We purified mAb2 from ascites fluid.

2.2. Nucleotide and amino acid sequences of monoclonal antibodies

The nucleotide and amino acid sequences of mouse anti human BChE monoclonal antibodies are deposited in the NCBI databank under accession numbers KJ141199 and KJ141200 for mAb2, KT189143 and KT189144 for B2 18–5, KT189145 and KT189146 for B2 12–1, and KT189147 and KT189148 for 11D8. Use of these monoclonal antibodies for immunoextraction of BChE from human plasma is described in Ref. [16].

2.3. BChE activity

BChE activity was measured in 0.1 M potassium phosphate pH 7.0 at 25 °C with 1 mM butyrylthiocholine iodide in the presence of 0.5 mM 5,5'-dithiobis(2-nitrobenzoic acid) on a Gilford spectrophotometer interfaced to a MacLab data recorder (ADinstruments, Inc.). For example, a 4 ml cuvette received 1.91 ml buffer, 0.05 ml of 20 mM DTNB in buffer, 0.02 ml of 0.1 M butyrylthiocholine dissolved in water, and 0.02 ml plasma. The increase in absorbance at 412 nm was recorded for 1 min and converted to micromoles butyrylthiocholine hydrolyzed using the extinction coefficient 13,600 M^{-1} cm^{-1} [21]. Units (U) of activity are expressed as micromoles per min.

2.4. Immunoadsorbance of BChE to monoclonal antibodies immobilized on pansorbin

Pansorbin® cells are an inexpensive alternative to Dynabeads-Protein G for assessing species selectivity of 5 monoclonal antibodies. Pansorbin® cells are heat-killed, formalin-fixed *Staphylococcus aureus* cells that bear a high cell-surface density of protein A.

The Pansorbin assay was performed as described by Brimijoin et al. [19]. The effectiveness of Pansorbin for binding mouse monoclonal antibodies is enhanced when Pansorbin cells are coated with rabbit anti-mouse IgG. Therefore, the first step in the protocol was to incubate 1 g of washed Pansorbin cells suspended in 9 ml of 50 mM TrisHCl pH 7.4 containing 0.1% BSA with 1 ml of 1 mg/ml rabbit anti-mouse IgG at 37 °C overnight. A 0.1 ml aliquot of rabbit anti-mouse IgG Pansorbin cell suspension was washed and incubated with 1 μg monoclonal in a total volume of 0.2 ml overnight. The control incubation contained Pansorbin cells and buffer, but no monoclonal. Unbound monoclonal was washed off. The washed Pansorbin complex was incubated overnight at room temperature on a rotating mixer with plasma or serum containing 13.5 milliunits of BChE supplemented with 50 mM TrisHCl pH 7.4/