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Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma

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Abstract

The goal of this work was to identify the esterases in human plasma and to clarify common misconceptions. The method for identifying esterases was nondenaturing gradient gel electrophoresis stained for esterase activity. We report that human plasma contains four esterases: butyrylcholinesterase (EC 3.1.1.8), paraoxonase (EC 3.1.8.1), acetylcholinesterase (EC 3.1.1.7), and albumin. Butyrylcholinesterase (BChE), paraoxonase (PON1), and albumin are in high enough concentrations to contribute significantly to ester hydrolysis. However, only trace amounts of acetylcholinesterase (AChE) are present. Monomeric AChE is seen in wild-type as well as in silent BChE plasma. Albumin has esterase activity with alpha- and beta-naphthylacetate as well as with *p*-nitrophenyl acetate. Misconception #1 is that human plasma contains carboxylesterase. We demonstrate that human plasma contains no carboxylesterase (EC 3.1.1.1), in contrast to mouse, rat, rabbit, horse, cat, and tiger that have high amounts of plasma carboxylesterase. Misconception #2 is that lab animals have BChE but no AChE in their plasma. We demonstrate that mice, unlike humans, have substantial amounts of soluble AChE as well as BChE in their plasma. Plasma from AChE and BChE knockout mice allowed identification of AChE and BChE bands without the use of inhibitors. Human BChE is irreversibly inhibited by diisopropylfluorophosphate, echothiophate, and paraoxon, but mouse BChE spontaneously reactivates. Since human plasma contains no carboxylesterase, only BChE, PON1, and albumin esterases need to be considered when evaluating hydrolysis of an ester drug in human plasma.

Keywords: Butyrylcholinesterase knockout mice; Acetylcholinesterase; Carboxylesterase; Paraoxonase; Human plasma; Albumin; Cocaine esterase; Aspirin; Irinotecan; Procaine; Succinylcholine; Mivacurium

1. Introduction

Esterases in human plasma have an important role in the disposition of drugs. They participate in activation of ester prodrugs, for example, the prodrug bambuterol is converted to the anti-asthma drug terbutaline, and isosorbide-based prodrugs release aspirin. A second role is to inactivate drugs. For example, esterases in plasma inactivate the local anesthetics procaine and tetracaine, the muscle relaxants, succinylcholine and mivacurium, and the analgesics, aspirin, and cocaine. A third role for esterases in plasma is to detoxify natural and synthetic ester-containing poisons, for example, eserine (physostigmine) from the Calabar bean and organophosphorus pesticides are detoxified by hydrolysis or by binding. Table 1 lists drugs modified by the action of esterases in human plasma. Note that aspirin is hydrolyzed by BChE and albumin, and that paraoxon is hydrolyzed by PON1 and albumin. Paraoxon also interacts with BChE, but is not listed in the BChE column because the binding is stoichiometric rather than catalytic. To understand individual variation in response to drugs it is important to know the identity of the esterase responsible for drug hydrolys is. A classic example is the case of the muscle relaxant succinylcholine. The era of pharmacogenetics was born when it was discovered that people who responded abnormally to succinylcholine had an inherited deficiency of

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Table 1
Clinically relevant compounds hydrolyzed by human plasma esterases

Drug action	BChE	PON1	Albumin	Reference
Analgesic	Aspirin		Aspirin	[58–60]
Analgesic	Isosorbide diaspirinate			[61]
Anti-asthma	Bambuterol			[62]
Local anesthetic, vasoconstrictor	(−)-Cocaine			[63,64]
Anti-cancer	Irinotecan (CPT-11)			[65]
Beta-blocker	Flestolol		Flestolol	[66]
Alpha-blocker, erectile dysfunction	Moxisylyte			[67]
Stimulates eating	*n*-Octanoyl ghrelin			[68]
Analgesic	Heroin			[69]
Local anesthetic	Procaine			[70]
Muscle relaxant	Succinylcholine			[2,71]
Muscle relaxant	Mivacurium			[72,73]
Anti-inflammatory	Methyl prednisolone acetate			[74]
Local anesthetic	Chloroprocaine			[75]
Local anesthetic	Tetracaine			[76]
Diuretic		Spironolactone		[77]
Lower cholesterol		Lovastatin, mevastatin, simvastatin		[77]
Pesticide		Paraoxon	Paraoxon	[51,78]
Pesticide		Diazoxon		[79]
Nerve agent		Sarin		[79]
Antibacterial		Prulifloxacin		[80]
Anti-asthma		Glucocorticoid-lactones		[81]
Anticholinesterase		Mono(diethylphosphoryl) obidoxime		[82]
Antabuse			Disulfiram	[83]
Anti-cancer			Cyclophosphamide	[84]
Anti-inflammatory			Ketoprofen glucuronide	[85]
Topical analgesic			Nicotinate esters	[16]
Pesticide			Carbaryl	[86]
Insecticide			*O*-Hexyl *O*-2,5-dichlorphenyl phosphoramidate	[17]

butyrylcholinesterase [1,2]. Today more than 56 mutations in the *BChE* gene have been identified [3]. People who have two deficient BChE alleles cannot metabolize succinylcholine and therefore are unable to breathe for 2 h from a dose intended to paralyze for 3–5 min.

Knowledge of the identity of the esterases involved in drug hydrolysis can help explain drug response in individuals with disease. For example, diabetics have higher than average BChE levels in their plasma [4–6] and might therefore require higher doses of aspirin for antiplatelet therapy to prevent stroke and myocardial ischemia.

A drug that requires the action of an esterase could be ineffective if that esterase were inhibited by another drug. For example, echothiophate eyedrops administered for treatment of glaucoma, inhibit plasma BChE [7]. Bambuterol might be ineffective as an asthma drug in a patient receiving echothiophate. Natural toxins present in food may also affect the metabolism of esters, and thus cause side effects. For instance, potato glyco-alkaloids (solanine and chaconine) that reversibly inhibit BChE have been reported to slow the degradation of the myorelaxant mivacurium [8].

While BChE, AChE, and PON1 are well known esterases, albumin is not usually included in the family of esterases. Albumin does not have an enzyme commission number, which signifies that this protein is considered to be inert without catalytic activity. However, albumin has been conclusively proven to be an esterase [9–20]. The active site of human albumin is Tyr 411 and of bovine albumin is Tyr 410 [21,22]. The active site of bovine albumin was identified by mass spectroscopy after labeling albumin with a biotinylated organophosphorus agent [22]. Although the enzymatic activity of a single molecule of human albumin is low, the concentration of albumin is very high, so that albumin makes a significant contribution to drug metabolism.

Our goal is to identify the esterases in human plasma, and to demonstrate the absence of carboxylesterase (EC 3.1.1.1) in human plasma.

2. Materials and methods

2.1. Materials

Silent BChE plasma samples were from the United States, India, and France. Silent plasma was stored at −20 °C. Silent BChE samples had no detectable activity with butyrylthiocholine or benzoylcholine. Human serum from people with wild-type BChE contained no anticoagulant and was stored at −80 °C. Serum containing wild-type BChE had an activity of 3–4 μmol/(min ml) (1 mM butyrylthiocholine, 0.1 M potassium phosphate buffer pH 7.0, 25 °C).

All mice were strain 129Sv. Mouse serum was freshly drawn from the hind leg vein. Gene-targeted BChE knockout mice (BChE−/−) (Li et al., unpublished) and AChE knockout mice (AChE−/−) were from colonies created and maintained at the University of Nebraska Medical Center [23,24].

Eserine, diisopropylfluorophosphate (DFP), butyrylthiocholine, acetylthiocholine, alpha-naphthylacetate, beta-naphthylacetate, Fast Blue RR, and human albumin fatty acid-free were from Sigma–Aldrich Corp., St. Louis, MO. Echothiophate was from Wyeth-Ayerst, Rouses Pt., NY. Paraoxon was from ChemService, West Chester, PA. Purified human BChE was prepared from outdated human plasma [25].

2.2. Nondenaturing gradient gel electrophoresis

Four to thirty percent polyacrylamide gels, 0.75 mm thick, were poured in a Hoefer gel apparatus. Electrophoresis was for 5000 V h (250 V for 20 h) at 4 °C. Plasma samples were mixed with an equal volume of 50% glycerol, 0.1 M Tris–Cl, pH 7.5, 0.1% bromophenol blue before loading on the gel. The equivalent of 5 μl of plasma or serum was loaded per lane.

2.3. Staining for AChE activity

The histochemical method of Karnovsky and Roots was adapted to polyacrylamide gels [26]. The staining solution contained 180 ml of 0.2 M maleic acid adjusted to pH 6.0 just before use, 15 ml of 0.10 M sodium citrate, 30 ml of 0.030 M $CuSO_4$, 30 ml water, 30 ml of 5 mM potassium ferricyanide, and 150 mg of acetylthiocholine iodide. Gels were incubated for 2–5 h, or overnight, with gentle shaking. Brown-red bands of activity developed.

2.4. Identification of AChE bands on gels

Gels stained with acetylthiocholine revealed both AChE and BChE because both enzymes have high activity with acetylthiocholine. Comparison to a gel stained with butyrylthiocholine, which stains only BChE, identified AChE. Silent BChE plasma had no BChE bands, but did have a band for AChE, thus confirming the identity of the AChE band in human plasma. AChE is inhibited by DFP, echothiophate, and paraoxon. The organophosphorus agents (OP) distinguish serine esterases from PON1 because PON1 is not inhibited by OP. Serum from knockout mice that had zero BChE activity allowed identification of AChE bands without the use of inhibitors.

2.5. Staining for BChE activity

The same Karnovsky and Roots staining solution was used to stain for BChE activity except that in place of acetylthiocholine, the substrate was 2 mM butyrylthiocholine iodide (180 mg in 300 ml).

2.6. Identification of BChE bands on gels

Bands of BChE activity were revealed on a gel in the presence of the BChE-specific substrate, butyrylthiocholine. AChE has only weak activity with butyrylthiocholine and does not stain, unless the amount of AChE is very high. Purified human BChE confirmed the identity of the tetramer BChE band in serum. Another confirmation was the absence of bands in plasma from people with silent BChE. Organophosphorus inhibitors, DFP, echothiophate, and paraoxon, distinguished between serine esterases and PON because serine esterases are inhibited, but PON is not inhibited by OP. Serum from knockout mice that had zero AChE activity allowed identification of BChE bands without the use of inhibitors.

2.7. Staining for PON1 activity

The method was adapted from [27]. The staining solution contained 100 ml of 50 mM Tris–Cl pH 8, 10 mM calcium chloride and 50 mg of beta-naphthylacetate dissolved in 1 ml ethanol. Some of the beta-naphthylacetate fell out of solution after addition to aqueous buffer, but this was not a problem. Solid Fast Blue RR, 50 mg, was added. Though Fast Blue RR did not dissolve, the reaction worked. Pink bands of activity formed within minutes. After 20–60 min, the gel was washed with water. Spots of dye were washed off with 50% methanol and 10% acetic acid.

2.8. Identification of PON1 on gels

Paraoxonase is a calcium-dependent enzyme associated with the high-density lipoprotein complex. PON stains with beta-naphthylacetate. PON is positively identified by its sensitivity to inhibition by 5 mM EDTA, when the EDTA is added to the plasma sample before gel electrophoresis. Blood collected into lavender cap tubes containing EDTA anticoagulant has no PON1 activity. Chelation of calcium inactivates PON. None of the other esterases in human and mouse plasma are inactivated by EDTA. The broad smear of PON activity on a gel is due to its association with high-density lipoprotein.

2.9. Staining for carboxylesterase activity

Staining with beta-naphthylacetate revealed PON1 as well as carboxylesterase activity. When it was desired to visualize carboxylesterase activity without PON1 activity, the substrate was alpha-naphthylacetate. Fifty milligrams of alpha-naphthylacetate in 1 ml ethanol and 50 mg of solid Fast Blue RR in 100 ml of 50 mM Tris–Cl pH 8 buffer gave dark green bands [28].

2.10. Identification of carboxylesterase on gels

Carboxylesterase stains well with alpha-naphthylacetate and with beta-naphthylacetate. Carboxylesterase is a serine esterase and is therefore inhibited by organophosphorus agents. However, carboxylesterase is distinguished from AChE and BChE by its lack of reactivity with positively charged substrates and positively charged OP [29]. Thus, carboxylesterase does not stain with the positively charged acetylthiocholine and butyrylthiocholine, and it is not inhibited by the positively charged echothiophate. Carboxylesterase is not inhibited by EDTA.

2.11. Staining for albumin esterase activity

Nondenaturing gels revealed albumin esterase activity when they were incubated in 100 ml buffer containing either alpha or beta-naphthylacetate (50 mg dissolved in 1 ml ethanol) and 50 mg of solid Fast Blue RR, for 10–60 min.

2.12. Identification of albumin on gels

Albumin esterase activity was identified by staining with alpha or beta-naphthylacetate. However, albumin showed no esterase activity with butyrylthiocholine or acetylthiocholine. Albumin esterase activity was resistant to inhibition by DFP, echothiophate, paraoxon, and EDTA. Albumin reacts covalently with OP at high pH and high concentrations of OP [9,30], but not under our experimental conditions. Gel electrophoresis of purified fatty acid-free human albumin confirmed the location of the albumin band on gels. During electrophoresis, the location of albumin is clearly seen as the slow migrating blue band in samples that contain bromophenol blue tracking dye. Bromophenol blue binds to human albumin. The bromophenol blue washes out of the gel during overnight incubation in staining solution.

2.13. Inhibitor treatment to identify esterases

To identify serine esterases, human and mouse plasma samples were incubated with 0.1 mM DFP, echothiophate, or paraoxon for 2 h at 25 °C, assayed for enzyme activity, and subjected to gel electrophoresis. In some cases, at the end of electrophoresis and before staining, the gels were pre-incubated in 0.1 mM OP in staining solution for 30 min before addition of substrate. To identify calcium-requiring esterase, plasma samples were pre-incubated with 10 mM EDTA.

2.14. Enzyme activity assays

BChE activity was measured with 1 mM butyrylthiocholine in 0.1 M potassium phosphate buffer pH 7.0 at 25 °C [31]. Combined AChE and BChE activity was measured with 1 mM acetylthiocholine.

3. Results

3.1. Human plasma contains four esterases

The esterases in human plasma are BChE, PON1, albumin, and AChE. The migration of each of these esterases relative to each other on nondenaturing gels is shown in Figs. 1–4. Fig. 1 shows the monomer, dimer,

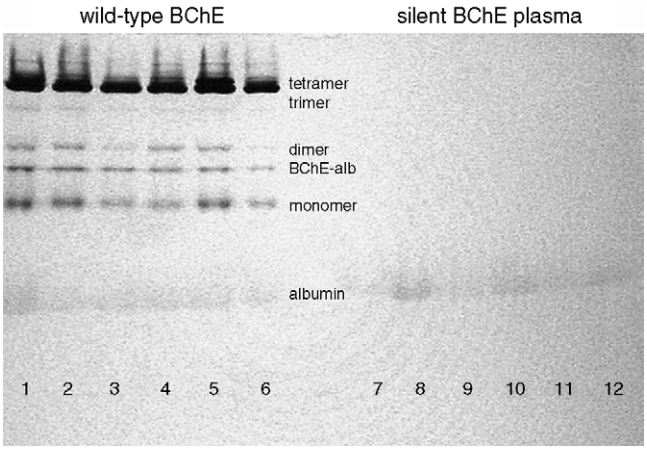

Fig. 1. Nondenaturing gel stained for BChE activity with butyrylthiocholine. Six wild-type BChE serum or plasma samples, and 6 silent BChE plasma samples from 12 different people were loaded at 5 μl per lane. Wild-type BChE samples show a predominance of BChE tetramers, and weak bands of BChE trimers, dimers, and monomers (lanes 1–6). Silent BChE samples are blank in corresponding regions (lanes 7–12). Albumin is identified by its transient binding of bromophenol blue. The band labeled BChE-alb is a disulfide-bonded dimer between one subunit of BChE and one molecule of albumin [92].

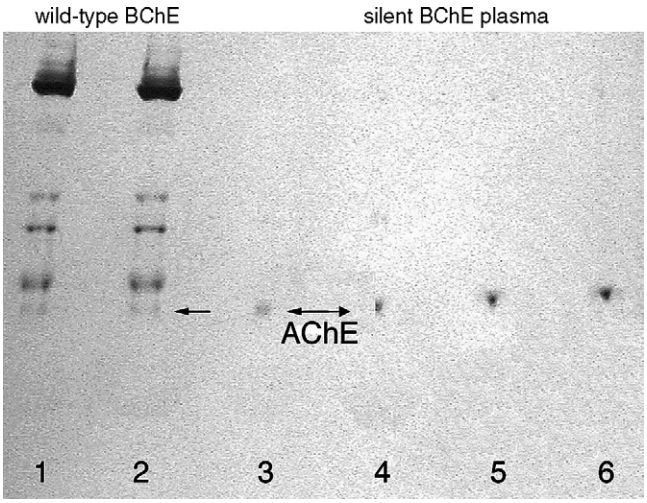

Fig. 2. Nondenaturing gel stained for activity with acetylthiocholine. A new band, not present in Fig. 1, appears below the BChE monomer band. This new band is AChE monomer. The AChE monomer is presented in wild-type (lanes 1 and 2) as well as in silent BChE human plasma (lanes 3–6). Human BChE tetramer, trimer, dimmer, and monomer bands are present in lanes 1 and 2. There is no albumin band on this gel because the bromophenol blue washed off. Two wild-type BChE sera were loaded at 5 μl per lane (lanes 1 and 2). One silent plasma was loaded at 5, 10, 15, and 20 μl per lane (lanes 3–6).

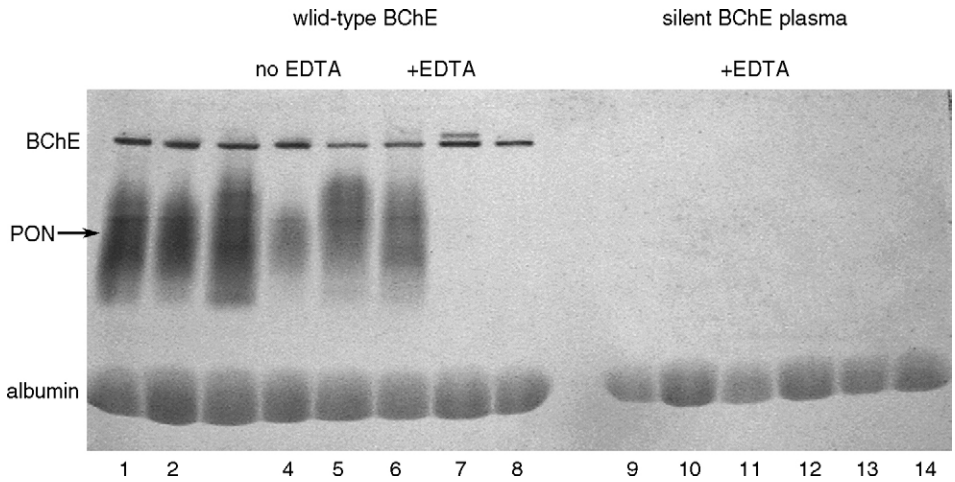

Fig. 3. Gel stained for PON activity with beta-naphthylacetate. Samples from 14 individuals were loaded on the gel: 6 serum samples contained wild-type BChE and no EDTA (lanes 1–6), 2 wild-type BChE samples with EDTA (lanes 7 and 9), and 6 silent BChE plasma samples with EDTA (lanes 9–14). The broad PON band is missing from all samples that contain EDTA. Albumin stains well with beta-naphthylacetate. The BChE tetramer shows up as a distinct band near the top of the gel. One wild-type sample contains a BChE doublet, representing the C5 BChE phenotype [38]. EDTA had no effect on BChE and albumin esterase activity.

trimer, and tetramer bands of BChE in human serum. Fig. 1 also shows that plasma from six individuals with silent BChE have no BChE activity as the lanes are blank. In Fig. 2, human plasma samples were run on a gel stained with acetylthiocholine, revealing a band of monomeric human AChE in silent BChE as well as in wild-type BChE samples. Fig. 3 shows PON activity in human serum and the absence of PON activity in EDTA-treated plasma. Figs. 3 and 4 show albumin esterase activity in gels developed with beta-naphthylacetate.

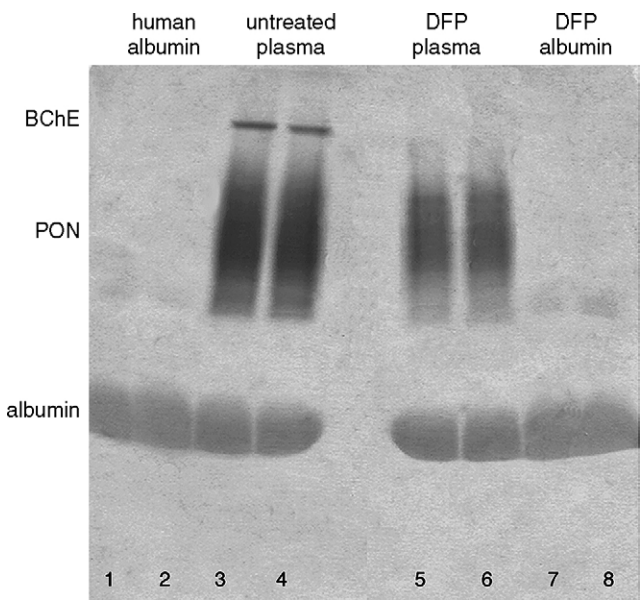

Fig. 4. Albumin esterase activity. The gel was stained with beta-naphthylacetate. Pure fatty acid-free human albumin is in lanes 1, 2, 7 and 8. Human plasma containing wild-type BChE is in lanes 3–6. Samples in lanes 1–4 were untreated. Samples in lanes 5–8 were treated with 0.1 mM DFP before electrophoresis. Pure fatty acid-free albumin (lanes 1 and 2) has the same band of activity as the albumin in untreated human plasma (lanes 3 and 4), thus confirming the identity of the albumin band. The intensity of the albumin band is unaffected by treatment with DFP under our experimental conditions. PON activity is also unaffected by treatment with DFP, but the BChE band disappears.

Albumin esterase activity stains equally well with beta- and alpha-naphthylacetate. Others have reported albumin esterase activity with naphthylacetate esters [13,32–34].

3.2. Sensitivity of detection

It has been estimated by immunochemical methods that human plasma contains 8 ng/ml of AChE [35,36]. A non-denaturing gel stained for AChE activity visualized a band of AChE activity in as little as 5 μl of human plasma, representing 0.04 ng of AChE.

Only monomeric AChE was detected, though tetrameric AChE has also been reported to be present in fresh human serum [36]. Silent BChE samples should have shown tetrameric AChE because silent BChE plasma has no interference from BChE. The absence of tetrameric AChE in silent BChE plasma is probably explained by the fact that plasma samples had been stored at −20 °C and freeze–thawed more than once, resulting in degradation of tetrameric AChE [37].

3.3. No carboxylesterase in human serum

Carboxylesterase migrates very close to albumin on nondenaturing gels. An intense band of activity with alpha-naphthylacetate is seen in the plasma of animals that have carboxylesterase, that is mouse, rat, rabbit, cat, tiger, and horse (Fig. 5). In contrast, man, monkey, chicken, cow, goat, sheep, and pig have no carboxylesterase enzyme in their plasma. A similar intense band of carboxylesterase activity is seen when the gel is stained with beta-naphthylacetate (data not shown).

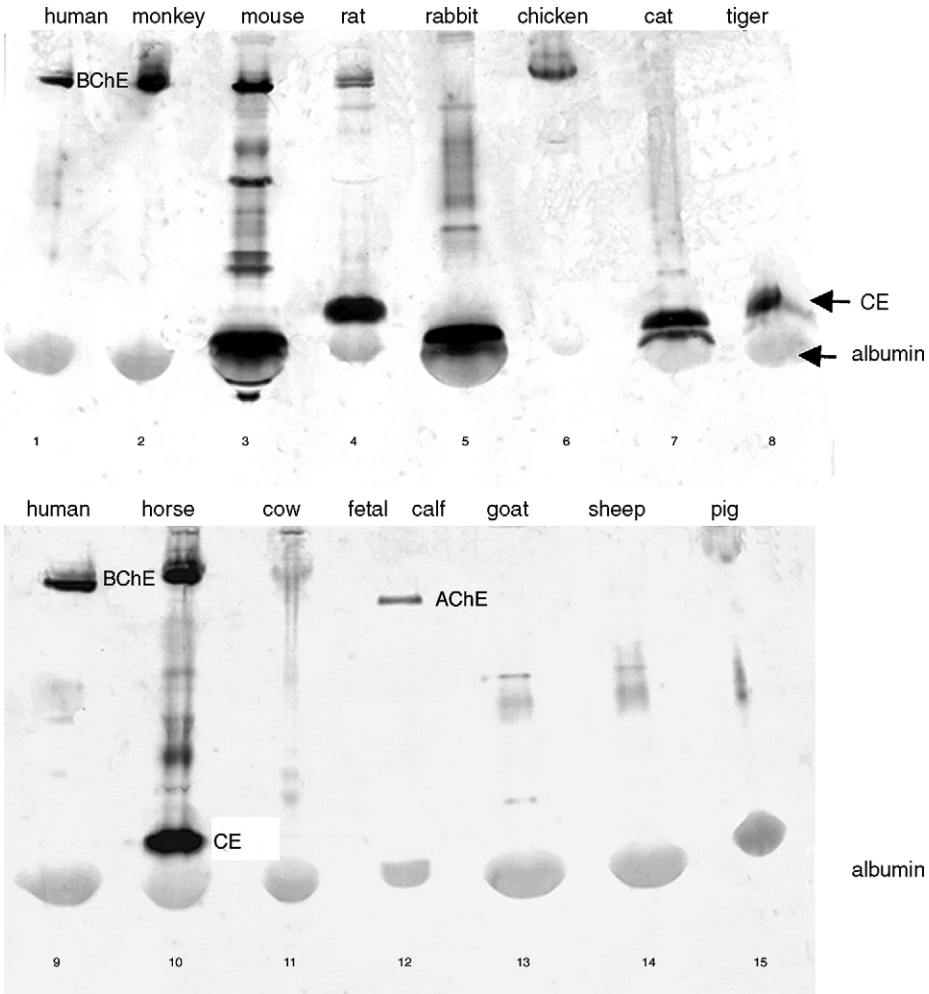

Fig. 5. No carboxylesterase in human serum. The nondenaturing gel was stained for activity with alpha-naphthylacetate. Five microliters of serum or plasma from each animal was loaded per lane. The intense band above albumin in mouse, rat, rabbit, cat, tiger, and horse is carboxylesterase (CE). Fetal calf but not adult cow serum has AChE (lanes 11 and 12).

The band was identified as carboxylesterase by its resistance to inhibition by EDTA, resistance to inhibition by the positively charged organophosphorus agent, echothiophate, and its sensitivity to inhibition by DFP and paraoxon. Resistance to inhibition by eserine, a specific cholinesterase inhibitor, was seen when the gel was pre-incubated with eserine before addition of alpha-naphthylacetate. None of the other esterases have all of these characteristics. In addition, we used mass spectroscopy to identify FP-biotin labeled carboxylesterase in mouse serum, and found that the FP-biotinylated carboxylesterase migrated on a nondenaturing gel just above albumin [30].

3.4. Visualizing AChE and BChE bands in knockout mouse plasma

Plasma from AChE knockout mice allowed clear identification of BChE bands, while plasma from BChE knockout mice allowed identification of AChE bands without the use of inhibitors. Fig. 6 compares the pattern of staining with acetylthiocholine for wild-type, BChE−/−, and AChE−/− mouse plasma. All the bands in lane 4 are AChE, while all the bands in lane 5 are BChE. The bands in lane 5 are darker than the bands in lane 4, consistent with activity assays which show 1.6 u/ml for BChE activity with acetylthiocholine in AChE−/− plasma and 0.2–0.5 u/ml for AChE activity in BChE−/− plasma. In lane 4, the heaviest band is an AChE tetramer. A weak band below the AChE tetramer band is at about the same level as the trimer band of BChE. AChE monomers and dimers are diffusely spread. The mouse BChE bands in lane 5 line up with some of the human BChE bands in lane 2. Mouse BChE tetramers, dimers, and monomers have corresponding BChE bands in human serum. However, the presumed BChE trimer in mouse serum migrates somewhat faster than the BChE trimer in human serum.

Use of inhibitors to reveal AChE and BChE bands gives the same general results, but obscures some of the detail because weak bands disappear.

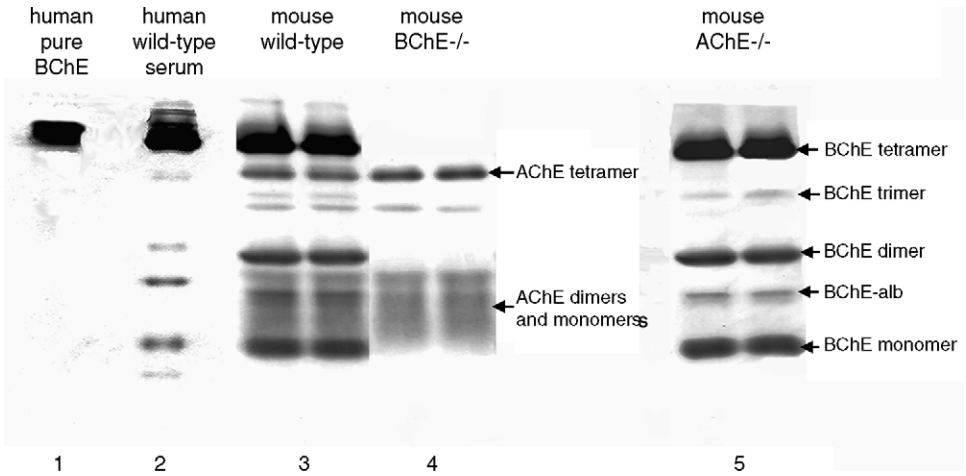

Fig. 6. BChE−/− and AChE−/− mouse plasma. The nondenaturing gel was stained with acetylthiocholine. Lane 4 containing BChE−/− mouse plasma, shows bands of AChE activity. Lane 5 containing AChE−/− mouse plasma, shows bands of BChE activity. For comparison, serum from wild-type mouse (lane 3), purified human BChE (lane 1), and wild-type human BChE serum (lane 2) are shown. BChE tetramers, dimmers, and monomers serve as markers for estimating the oligomers of AChE.

3.5. Storage bands

Serum or plasma stored at −20 °C has bands of BChE not seen in freshly drawn blood or in blood stored at −80 °C. These bands have been referred to as storage bands [38]. Fig. 7 illustrates storage bands in mouse serum. The storage bands are seen as three new bands migrating more slowly than tetrameric BChE, as well as new bands and more intense bands in the dimer region. Broad fuzzy bands appear in the region between dimers and tetramers. Storage bands are probably caused by desialylation and proteolysis [39].

Note the slow-migrating thin doublet in lane 2 of Fig. 6 for wild-type human serum. These minor bands of MW approximately 900 kDa are different from storage bands; they correspond to monomeric BChE bound to α2-macroglobulin doublet (Masson, unpublished result). Not all individuals show the 900 kDa bands.

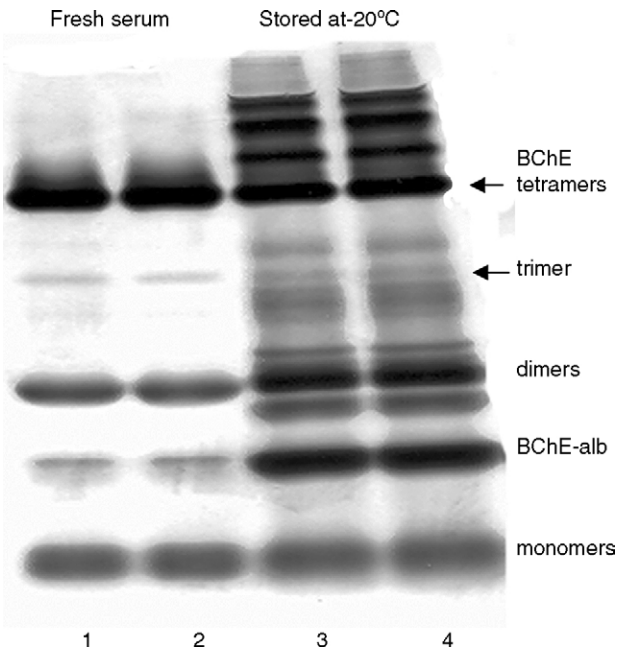

Fig. 7. Storage bands in mouse serum. The nondenaturing gel was stained for activity with butyrylthiocholine to show BChE bands. Lanes 1 and 2 contain fresh serum from a wild-type mouse. Lanes 3 and 4 contain mouse serum stored for 2 years at −20 °C.

3.6. Unidentified bands of esterase activity in mouse plasma

On nondenaturing gels stained with alpha-naphthylacetate, mouse plasma had four novel bands. These bands were neither BChE nor AChE because they were present in AChE−/− as well as in BChE−/− plasma (see Fig. 8). These bands completely disappeared when the gel was pre-incubated in 0.1 mM DFP for 30 min before staining. The carboxylesterase and cholinesterase bands also disappeared, suggesting that the unknown esterases could be serine esterases.

3.7. Spontaneous reactivation of OP-inhibited mouse BChE

Mouse BChE spontaneously reactivated after inhibition by OP, but human BChE did not. The mouse and human plasma samples in Fig. 9 were treated with 0.1 mM DFP, echothiophate, or paraoxon for 2 h, assayed for activity with acetylthiocholine, and then loaded on a nondenaturing gel. After 20 h of electrophoresis at 4 °C, the gel was stained for activity with acetylthiocholine. The surprising result in Fig. 9 was the presence of BChE activity in samples treated with OP.

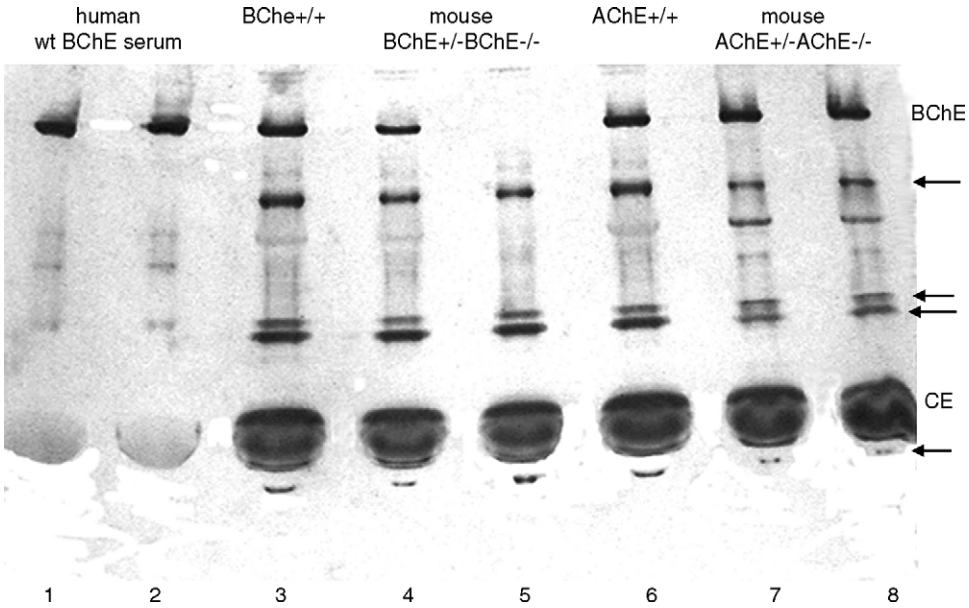

Fig. 8. Four unidentified esterase bands. The gel was stained with alpha-naphthylacetate. Five microliters of serum from two humans (lanes 1 and 2), and from six mice were loaded per lane (lanes 3–8). The mice were wild-type (lane 3), heterozygous for the BChE knockout (lane 4), homozygous for the BChE knockout (lane 5), wild-type (lane 6), heterozygous for the AChE knockout (lane 7), and homozygous for the AChE knockout (lane 8). The four unidentified esterase bands are indicated with an arrow. The heaviest staining is for mouse carboxylesterase (CE).

These samples had shown no cholinesterase activity before they were loaded on the gel. We conclude that some of the OP spontaneously detached from BChE during the 20 h of electrophoresis. Excess OP separated from enzyme on the gel, allowing the reactivated BChE to stay active. Reactivation after DFP phosphorylation was weaker than for enzyme inhibited by echothiophate and paraoxon, because aging of the diisopropylphosphate-adduct competed with reactivation.

In another experiment (data not shown), the gel was pre-incubated in 0.1 mM DFP, 0.1 mM echothiophate, or 0.1 mM paraoxon for 30 min before being stained for activity. Under these conditions, BChE enzyme activity was completely inhibited.

Human BChE did not spontaneously reactivate after treatment with 0.1 mM DFP, echothiophate, or paraoxon. Spontaneous reactivation of OP-inhibited mouse BChE has also been reported by others [40–42].

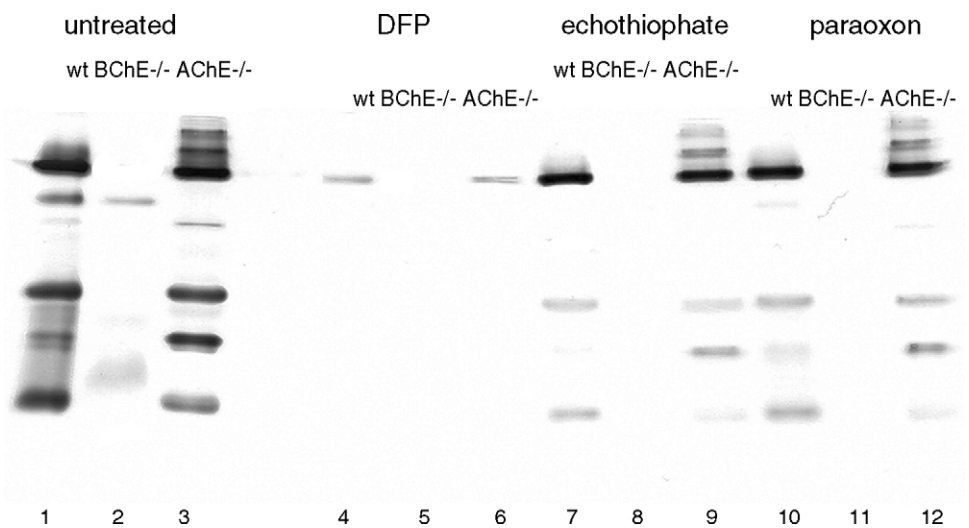

Fig. 9. Spontaneous reactivation of OP-inhibited mouse BChE. The gel was stained for activity with acetylthiocholine. Mouse sera from wild-type, BChE knockout and AChE knockout mice were untreated (lanes 1–3), or incubated with 0.1 mM DFP (lanes 4–6), 0.1 mM echothiophate (lanes 7–9), or 0.1 mM paraoxon (lanes 10–12) before being loaded on the gel. The BChE in wild-type (lanes 4, 7, and 10) and AChE−/− sera (lanes 6, 9, and 12) regained some activity during the 20 h electrophoresis run, after being completely inhibited by OP. However, OP treated sera from BChE−/− mice have blank lanes (lanes 5, 8, and 11), indicating that AChE activity was irreversibly inhibited.

4. Discussion

4.1. Three esterases in human plasma

The convention of naming an esterase for the drug or substrate being studied gives the impression that human plasma contains dozens of different esterases. However, we find only four esterases in human plasma, and one of these, AChE, is present in negligible amounts. This leaves only BChE, PON1, and albumin to carry out ester hydrolysis.

Our analysis assumes that any esterase present in significant quantity would have been stained with alpha or beta-naphthylacetate. This assumption is verified by searching the proteomics databases.

4.2. Proteomics

New mass spectroscopy tools have allowed investigators to undertake the project of identifying all the proteins in human plasma. The Plasma Proteome Institute suggests that human plasma may contain most, if not all, human proteins, though many will be present at very low concentrations. In the year 2004, the number of nonredundant distinct gene products in human plasma was 1175 [43]. In the year 2005, this number had grown to 3778 [44]. The lists include butyrylcholinesterase, paraoxonase, and albumin, but not acetylcholinesterase or carboxylesterase. The absence of acetylcholinesterase from the list suggests that a low abundance protein, present at a concentration no higher than 0.005 mg/l, is not easily detected by mass spectroscopy.

4.3. No carboxylesterase in human plasma

The idea that human plasma contains carboxylesterase [41,45–48] comes from the fact that carboxylesterase activity is assayed with *p*-nitrophenyl acetate, and this same substrate is also hydrolyzed by BChE and albumin. PON1, called arylesterase and A esterase in the earlier literature, also has activity with *p*-nitrophenyl acetate [49–51]. After BChE activity is inhibited with eserine or *iso*-OMPA, the residual *p*-nitrophenyl acetate activity has been erroneously attributed to carboxylesterase, whereas it is our contention that the residual activity comes from albumin and PON1. Albumin esterase activity is routinely assayed with *p*-nitrophenyl acetate [9,10,14].

Our finding that human plasma contains no carboxylesterase is confirmed by the reports of others who also find no carboxylesterase in human plasma [52,53]. Aliesterase, an early name for carboxylesterase, was reported to be absent in human, monkey, pig, ruminant, and chicken plasma, consistent with our finding [54]. We cannot rule out the presence of a negligible amount of carboxylesterase, too low to detect by our methods, and too low to detect by mass spectrometry techniques. We did not find carboxylesterase listed in any human proteomics databank. Since acetylcholinesterase is also absent from these lists, yet we know AChE is present, we can conclude that if carboxylesterase is present, its concentration is in the same low range as AChE.

We cannot rule out the possibility that under specific pathological conditions significant amounts of carboxylesterase may be released into the blood circulation.

4.4. Mouse plasma

The esterases in mouse plasma are BChE, PON, albumin, AChE, and carboxylesterase. In addition, there are four OP-sensitive bands whose identity is not yet known. The amount of AChE in mouse plasma is 25-fold higher than in human plasma (see Table 2). Carboxylesterase is 30-fold more abundant than BChE. The high amount of carboxylesterase in rodent and rabbit plasma has made these animals inappropriate models for drug and OP effects in humans. Only after carboxylesterase was inactivated by treatment with cresylbenzodioxaphosphorin oxide did interspecies differences disappear with regard to soman toxicity [55]. The pharmacokinetic profile for irinotecan in carboxylesterase deficient mice more closely reflected that seen in humans [53].

4.5. Whole blood

Whole blood contains additional esterases not described in this report. The short duration of action of esmolol, a very short-acting beta1-adrenoceptor antagonist, is attributed to rapid enzymatic hydrolysis by esterases in the cytosol of red blood cells [56]. This esterase could be Esterase D (also called *S*-formylglutathione hydrolase) since Esterase D is known to be present in the red blood cell cytosol. Another esterase in red blood cells is acetylcholinesterase. AChE is attached to the outer surface of the red cell membrane via a glycolipid anchor. Though AChE is inhibited by anti-Alzheimer disease drugs, AChE is not known to participate in hydrolysis of drug esters.

Table 2
Esterases in human and mouse plasma

Esterase	Human plasma (mg/l)	Mouse plasma (mg/l)
BChE (EC 3.1.1.8)	5	2.6
AChE (EC 3.1.1.7)	0.008	0.2
PON1 (EC 3.1.8.1)	50	25
Albumin	50000–60000	50000–60000
Carboxylesterase (EC 3.1.1.1)	0	80

BChE activity in u/ml was converted to mg/l by using a specific activity of 720 u/mg for butyrylthiocholine. The value for AChE in human plasma is from [35]. AChE activity in mouse plasma was converted to mg/l by using a specific activity of 2600 u/mg for acetylthiocholine. The value for PON1 in human plasma is from [87,88]. The value for PON in mouse plasma was calculated from [89]. Albumin concentration is from [90]. The value for carboxylesterase in mouse plasma was calculated from a specific activity of 228 u/mg with *p*-nitrophenyl acetate [91] and an activity of 18.6 u/ml [30].

Complement component C1s is found in plasma at a concentration of 50 mg/l, but it must be proteolytically activated by an antigen–antibody complex before it has protease and esterase activity [57]. Platelets and lymphocytes contain neuropathy target esterase, while T-lymphocytes contain ubiquitin carboxyl terminal esterase L1 [44]. These esterases are present in low abundance and have not been reported to have a role in hydrolysis of drug esters.

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