Effects of simvastatin and fenofibrate on butyrylcholinesterase activity in the brain, plasma, and liver of normolipidemic and hyperlipidemic rats

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The study objective was to test the hypothesis that simvastatin and fenofibrate should cause an increase in butyrylcholinesterase (BuChE) activity not only in the plasma and liver but also in the brain of normolipidemic and hyperlipidemic rats. Catalytic enzyme activity was measured using acetylthiocholine (ATCh) and butyrylthiocholine (BTCh) as substrates. Normolipidemic and hyperlipidemic rats were divided in four groups receiving 50 mg/kg of simvastatin a day or 30 mg/kg of fenofibrate a day for three weeks and three control groups receiving saline. Simvastatin and fenofibrate caused an increase in brain BuChE activity in both normo- and hyperlipidemic rats regardless of the substrate. The increase with BTCh as substrate was significant and practically the same in normolipidemic and hyperlipidemic rats after simvastatin treatment (14–17% *vs* controls). Simvastatin and fenofibrate also increased liver and plasma BuChE activity in both normolipidemic and hyperlipidemic rats regardless of the substrate. In most cases the increase was significant. Considering the important role of BuChE in cholinergic transmission as well as its pharmacological function, it is necessary to continue investigations of the effects of lipid-lowering drugs on BuChE activity.

KEY WORDS: acetylthiocoline; butyrylthiocholine; lipid-lowering drugs; Wistar rats; Zucker rats

Butyrylcholinesterase (BuChE), also known as pseudocholinesterase (PChE) and plasma cholinesterase (EC 3.1.1.8), is synthesised in the liver and secreted into the blood circulation (1, 2). BuChE is found in the blood, pancreas, liver, central nervous system, and other tissues, but its function and endogenous substrate have not yet been established (3). Some authors propose that increased plasma BuChE activity in humans is associated with an abnormal lipid metabolism (1, 2, 4, 5). This enzyme is biochemically related to acetylcholinesterase (AChE, EC 3.1.1.7), and although it is less substrate-specific for acetylcholine (ACh) than AChE, BuChE is the major ACh-hydrolysing enzyme in the circulation (6). In the brain, BuChE is involved in ACh hydrolysis together with AChE and serves as a co-regulator of cholinergic transmission. BuChE also hydrolyses a variety of other choline esters including butyrylcholine (BCh), propionylcholine, succinylcholine, and the aminoester local anaesthetics. According to new research (7), this enzyme is also involved in the degradation of ghrelin, a hormone responsible for the sensation of hunger.

Different drugs may either decrease or increase the activity of BuChE and AChE. Inhibition of plasma BuChE in rats and humans may be caused by organophosphorus herbicides such as glyphosate (8), reversible cholinesterase inhibitors (9), glucocorticoids (10, 11), or some beta blockers (12). In humans, low plasma BuChE activity may reduce succinylcholine hydrolysis and prolong respiratory depression (13). In contrast, brain inhibition of BuChE and AChE activity may be beneficial for the therapy of older population, as the inhibitors of both enzymes enhance the activity of cholinergic neurons in the brain, which is of particular benefit in patients with Alzheimer's disease, who have decreased forebrain cholinergic neuron count and a progressive decrease in ACh (14, 15).

Like many other, lipid-lowering drugs affect enzyme activity. Several *in vitro* and *in vivo* non-clinical investigations have evidenced that statins either decrease or have no effect on AChE and BuChE activity (16, 17). Our recent study, however, has shown that simvastatin significantly increases plasma and liver BuChE activity in normolipidemic rats (18). Fibrates have also been shown

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to increase BuChE activity in non-clinical studies (19, 20). Clinical evidence, in turn, is still not strong enough to reach definitive conclusions about the effects of lipid-lowering agents on plasma BuChE activity (4, 21).

Because of the pharmacological function of BuChE in plasma, its role in the brain cholinergic system, and the widespread therapeutic use of lipid-lowering drugs, we extended our investigation to include the effect of lipid-lowering agents on brain BuChE activity and test the hypothesis that they should increase brain BuChE activity like they do in the plasma and liver. Simvastatin and fenofibrate as representatives of statins and fibric acid derivatives were chosen for this investigation because both agents cross the blood-brain barrier. We also wanted to see if the increase in brain, plasma, and liver enzyme activity would be greater in hyperlipidemic rats.

MATERIALS AND METHODS

Test substances

Simvastatin (CAS-79902-63-9) (Lipex®, Merck Sharp & Dohme, Haarlem, the Netherlands) and fenofibrate (CAS-49562-28-9) (Tricor®, Recipharm Fontaine, Fontaine-lès-Dijon, France) tablets were suspended in 5 mL/kg saline and administered daily between 9.00 and 10.00 a.m. by oral gavage.

Animals

Normolipidemic Wistar rats weighing 250–300 g were obtained from the Department of Pharmacology, University of Zagreb School of Medicine, Croatia, whereas the hyperlipidemic Zucker rats (HsdOla: Zucker-Lepr fa-fa) weighing 270–300 g were obtained from Harlan Laboratories srl., Udine, Italy. The animals were kept under controlled laboratory conditions and receiving standard pelleted diet *ad libitum*. Handling and treatment followed the FELASA guidelines and recommendations for the use of laboratory animals (22). The experiments had been approved by the local committee of the Zagreb University School of Medicine.

Study design

Normolipidemic rats were randomly divided in two experimental (N=10 each) and two control groups (N=8 each). One experimental group received 50 mg/kg of simvastatin a day and the other 30 mg/kg of fenofibrate a day. Control groups received 0.9% NaCl solution, as it was solvent for drugs in the experimental groups. The route of administration was the same as in the experimental groups (oral gavage). Hyperlipidemic Zucker rats were also randomly divided in two experimental and one control group. One experimental group (N=7) received simvastatin and the other (N=8) fenofibrate in the same doses and way

as the normolipidemic rats. The control group (N=6) received saline as above.

The experimental treatment lasted 21 days. On day 22, after a 12-hour overnight fast, all animals were sacrificed under diethyl ether anaesthesia (23). Blood samples for the measurement of BuChE activity were obtained by cardiac puncture and frozen at -20 °C immediately after sampling until further processing. Liver and brain tissue for the determination of BuChE activity was frozen at -70 °C. Before collecting, blood was washed from the liver with saline *in situ* through the superior vena cava. The brain was also rinsed with saline.

Measurement of BuChE activity in the brain, plasma, and liver

The catalytic activity of BuChE in the brain, plasma, and liver was measured spectrophotometrically as described by Ellman et al. (24) using butyrylthiocholine (BTCh) (0.9 mmol/L) and acetylthiocholine (ATCh) (0.6 mmol/L) as substrates (Sigma ChemCo, St. Louis, MO, USA). Whole brains weighing 1.8 to 2.2 g and small portions of liver weighing 0.7 to 0.98 g were homogenised in four volumes of saline and centrifuged at 3500 x *g* for 15 min. The reaction mixture for the hydrolysis in the brain, plasma, and liver contained 1 mL of a mixture of 3 mL 0.1 mol phosphate buffer and 100 µL of 0.38 mmol/L 5.5-dithio-bis (2-nitrobenzoic acid) (DTNB), 100 µL of BTCh or 100 µL of ATCh, and 50 µL of plasma or 50 µL of brain or liver homogenate. The reaction for the measurement of BuChE activity in the liver tissue was repeated after the addition of 50 µL of ethopropazine hydrochloride, which is a specific BuChE inhibitor. The increase in absorbance at 412 nm and at 25 °C was monitored for 3 min. A blank sample containing the incubation mixture without the brain, plasma, and liver homogenate was run simultaneously to correct for the spontaneous substrate breakdown. Enzyme activities were expressed as µmol/L of substrate hydrolysed per min per mL of plasma or g of tissue.

Data analysis

Data were analysed using Dunnett's multiple comparison test, which compares treatment groups with controls (25). All tests were two-tailed, and the P values of <0.05 were considered statistically significant. All calculations and data plotting were done using GraphPad Prism for Windows version 7 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Brain, plasma, and liver BuChE activity in normolipidemic rats

Table 1 shows brain, plasma, and liver BuChE activity in normolipidemic rats. Brain BuChE activity measured with ATCh was not significantly affected by simvastatin

Table 1 Effects of simvastatin (50 mg/kg daily for three weeks) and fenofibrate (30 mg/kg daily for three weeks) on brain, plasma, and liver BuChE activity in normolipidemic rats, measured with ATCh and BTCh as substrates

Substrate	Variable	Control[a]	Simvastatin[a] (50 mg/kg/day)	P Value[b] (95% CI)[c]	Control[a]	Fenofibrate[a] (30 mg/kg/day)	P Value[b] (95% CI)[c]
ATCh	BuChE brain (µmol/min/g)	0.29±0.018 (n=8)	0.28±0.006 (n=8)	0.477 (-0,019 to 0,009)	0.37±0.035 (n=8)	0.35±0.016 (n=8)	0.412 (-0.047 to 0.021)
	BuChE plasma (µmol/min/mL)	0.31±0.063 (n=8)	0.38±0.048 (n=7)	**0.029** (0.009 to 0.134)	0.25±0.049 (n=7)	0.37±0.073 (n=8)	**0.003** (0.051 to 0.195)
	BuChE liver (µmol/min/g)	0.58±0.165 (n=8)	0.69±0.207 (n=7)	0.263 (-0.095 to 0.319)	0.47±0.243 (n=6)	0.86±0.150 (n=5)	**0.013** (0.106 to 0.674)
BTCh	BuChE brain (µmol/min/g)	0.06±0.009 (n=8)	0.07±0.002 (n=8)	**0.043** (0.001 to 0.014)	0.08±0.006 (n=8)	0.09±0.003 (n=8)	0.167 (-0.002 to 0.009)
	BuChE plasma (µmol/min/mL)	0.07±0.010 (n=8)	0.11±0.005 (n=8)	**<0.001** (0.022 to 0.049)	0.08±0.014 (n=8)	0.13±0.038 (n=6)	**0.003** (0.022 to 0.085)
	BuChE liver (µmol/min/g)	0.79±0.292 (n=6)	0.82±0.177 (n=5)	0.871 (-0.314 to 0.364)	0.41±0.284 (n=8)	0.76±0.115 (n=7)	**0.009** (0.108 to 0.606)

Bolded P values are significant at the <0.05 level; ATCh – acetylthiocholine, BTCh – butyrylthiocholine, BuChE – butyrylcholinesterase; [a] values are mean ± SD; [b] P value of independent two-sample t-test; [c] 95% confidence interval for difference between means

and fenofibrate. In plasma its activity increased 22% (p=0.029) with simvastatin and 48% (p=0.003) with fenofibrate vs control (Table 1). In the liver BuChE catalytic activity soared 83% (p=0.013) with fenofibrate vs control, while the increase of 19% with simvastatin was not statistically significant (Table 1).

When BTCh was used as substrate, simvastatin increased brain BuChE activity 17% *vs* control (p=0.043), and fenofibrate caused only mild changes. In the plasma, BuChE activity increased 57% with simvastatin (p<0.001) and 63% with fenofibrate (p=0.003). Liver BuChE activity increased 85% (p=0.009) with fenofibrate, while simvastatin caused only a mild increase.

Brain, plasma, and liver BuChE activity in hyperlipidemic rats

Table 2 shows brain, plasma, and liver BuChE activity in hyperlipidemic rats. Measured with ATCh as substrate brain BuChE activity increased 18% *vs* control (p=0.008) with simvastatin and 7% with fenofibrate (p>0.05). Plasma BuChE activity increased 56% (p<0.001) and 84% (p<0.001), whereas liver BuChE activity increased 40% (p<0.001) and 59% (p<0.001) vs control, respectively.

With BTCh used as substrate brain BuChE activity increased 14% (p<0.001) with both simvastatin and fenofibrate. Plasma BuChE activity increased 94% with simvastatin and as high as 144% with fenofibrate (p<0.001), whereas liver BuChE activity increased 103% (p=0.003) with simvastatin and only 26% with fenofibrate (p>0.05).

Our results confirm our hypothesis that simvastatin and fenofibrate would increase brain BuChE activity in both normolipidemic and hyperlipidemic rats regardless of the type of substrate used to measure enzyme activity. The exception is brain BuChE activity in normolipidemic rats when ATCh was used as substrate. The reason is higher affinity of BuChE for BTCh than for ATCh, i.e. the enzyme is less substrate-specific for ACh. Since BuChE is biochemically related to AChE, we believe that this difference in substrate sensitivity is not an obstacle to interpret our results in light of reports of AChE activity. Cibicková et al. (17) were the first to show the inhibitory effect of simvastatin and atorvastatin on brain AChE activity in the frontal cortex of rats. Their second study (26), in contrast, found no change in brain AChE activity. Dalla et al. (27) also reported that simvastatin and pitavastatin had no effect on brain AChE activity in rats on normal diet. These studies and our investigations suggest that statins have different effect on BuChE and AChE activity.

We cannot, however, interpret the fenofibrate-induced increase in brain BuChE activity in light of other studies, as none have been published so far. Fenofibrate showed similar effects to simvastatin on brain BuChE activity, i.e. increase in its activity, as it causes a faster hydrolysis of ACh and consequently affects brain cholinergic transmission. In patients on long-term statin or fibrate therapy, the increase in brain BuChE activity could therefore cause side effects.

Simvastatin and fenofibrate in our study also caused higher liver and plasma BuChE activity in both rat strains regardless of the used substrate (Tables 1 and 2), but the

Table 2 Effects of simvastatin (50 mg/kg daily for three weeks) and fenofibrate (30 mg/kg daily for three weeks) on brain, plasma, and liver BuChE activity in hyperlipidemic rats, measured with ATCh and BTCh as substrates

Substrate	Variable	Control[a]	Simvastatin[a] (50 mg/kg/day)	P Value[b] (95% CI)[c]	Fenofibrate[a] (30 mg/kg/day)	P Value[b] (95% CI)[c]
ATCh	BuChE brain (µmol/min/g)	0.27±0.020 (n=6)	0.32±0.038 (n=6)	**0.008** (-0.079 to -0.012)	0.29±0.005 (n=7)	0.272 (-0.052 to 0.013)
	BuChE plasma (µmol/min/mL)	0.43±0.025 (n=6)	0.67±0.096 (n=6)	**<0.001** (-0.363 to -0.104)	0.79±0.118 (n=8)	**<0.001** (-0.475 to -0.233)
	BuChE liver (µmol/min/g)	1.85±0.237 (n=6)	2.60±0.152 (n=6)	**<0.001** (-1.044 to -0.459)	2.95±0.226 (n=8)	**<0.001** (-1.376 to -0.828)
BTCh	BuChE brain (µmol/min/g)	0.07±0.004 (n=6)	0.08±0.006 (n=5)	**<0.001** (-0.021 to -0.009)	0.08±0.003 (n=8)	**<0.001** (-0.018 to -0.007)
	BuChE plasma (µmol/min/mL)	0.16±0.011 (n=6)	0.31±0.043 (n=6)	**<0.001** (-0.203 to -0.079)	0.39±0.06 (n=8)	**<0.001** (-0.287 to -0.172)
	BuChE liver (µmol/min/g)	0.38±0.193 (n=6)	0.77±0.147 (n=6)	**0.003** (-0.646 to -0.148)	0.48±0.189 (n=8)	0.455 (-0.339 to 0.127)

Bolded P values are significant at the <0.05 level; ATCh – acetylthiocholine, BTCh – butyrylthiocholine, BuChE – butyrylcholinesterase; [a] values are mean ± SD; [b] P value of independent two-sample t-test; [c] 95% confidence interval for difference between means

effect was mostly stronger in hyperlipidemic rats. In some cases BuChE activity was higher when BTCh was used as substrate (Table 1), especially in hyperlipidemic rats (Table 2). A possible reason why simvastatin and fenofibrate should cause higher enzyme activity in the plasma and liver of hyperlipidemic rats is their pathophysiological condition.

Higher BuChE activity in this study confirms our previous findings for simvastatin and gemfibrozil, the other member of fibric acid derivatives, as well as the results reported by other authors who showed that clofibrate, gemfibrozil, and fenofibrate significantly increased serum and liver BuChE activity in rats and mice (28–30). In contrast, Cibicková et al. (26) did not prove any influence of simvastatin and atorvastatin on either AChE or BuChE in the blood.

The significant increase in liver and plasma BuChE activity caused by simvastatin and fenofibrate is likely a consequence of the higher expression of peroxisome proliferator-activated receptor alpha (PPARα), which is a well known transcriptional factor (31–33). In rodents PPARα is primarily expressed in the liver and adipose tissue and, to a lesser extent, in the kidney, heart, and skeletal muscles. It plays an important role in lipid homeostasis, as it influences fatty acid metabolism and its activation lowers lipid levels in animals and humans (34–36).

According to Ferreira et al. (37), fenofibrate treatment increased PPAR alpha and gamma receptors in rat adipose tissue and in consequence of their activation also increased the expression of some oxidative enzymes. It had the same effect on oxidative enzymes in rat liver (37–39). This

suggests that the activation of PPARα receptor also stands behind fenofibrate increasing BuChE expression in the liver.

PPARα receptors have recently been reported in some brain regions, where they probably have an important role in brain lipid metabolism (40, 41). Berger et al. (42) have also suggested that specific fatty acids that play a crucial role in neural cells and are essential components of myelin are synthesised in peroxisomes. Further research should answer whether higher brain BuChE activity in our study is partly owed the agonistic effects of simvastatin and fenofibrate on brain PPARα receptors and on these special fatty acids.

It is known for a fact that rodents are more susceptible to peroxisome proliferators than dogs, rhesus monkeys, or humans. Probably this difference in PPARα receptor sensitivity to statins and fibrates is the reason why statins either do not affect or lower plasma enzyme activity in humans (4, 21, 43).

Clinical investigations referred to above lasted no longer than six months, which begs the question whether long-term lipid-lowering therapy could change the sensitivity of PPARα and cause an increase rather than a decrease in BuChE activity in human patients.

In summary, our results clearly show that simvastatin and fenofibrate cause a similar increase in brain BuChE activity in normolipidemic and hyperlipidemic rats. Such increases in brain BuChE activity could lead to side effects in patients on long-term therapy with statins and fibrates. We have also verified our earlier findings that simvastatin and fibric acid derivatives increase liver and plasma BuChE activity. However, it is hard to extrapolate our findings in

humans at this point, because rats are more susceptible to peroxisome proliferators than humans. Future pre-clinical research should therefore focus on the molecular mechanisms of these changes. Because of the pharmacological role of BuChE in plasma and brain cholinergic system, long-term clinical studies are also needed to elucidate the impact of statins and fibrates on BuChE activity.

Conflicts of interests

None to declare.

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Učinci simvastatina i fenofibrata na aktivnost butirilkolinesteraze u mozgu, plazmi i jetri normolipidemičnih i hiperlipidemičnih štakora

Cilj ispitivanja bio je u normolipidemičnih i hiperlipidemičnih štakora potvrditi pretpostavku da simvastatin (SIMV) i fenofibrat (FENO) ne uzrokuju samo povećanje aktivnosti butirilkolinesteraze (BuChE) u plazmi i jetri nego i povećavaju aktivnost BuChE u mozgu. Katalitička aktivnost enzima mjerena je upotrebom acetiltiokolina (ATCh) i butiriltiokolina (BTCh) kao supstrata. Normolipidemični i hiperlipidemični štakori raspoređeni su u eksperimentalne skupine, koje su tri tjedna primale SIMV 50 mg/kg na dan ili FENO 30 mg/kg na dan, dok su kontrolne skupine primale fiziološku otopinu. I SIMV i FENO uglavnom su izazvali povećanje aktivnosti BuChE u mozgu obaju sojeva štakora bez obzira na korišteni supstrat. Aktivnosti BuChE mjerene BTCh kao supstratom bile su značajno veće u odnosu na kontrolne vrijednosti u mozgu normolipidemičih štakora nakon primjene SIMV te u mozgu hiperlipidemičnih štakora nakon primjene obaju agensa (14–17%, p<0,001). Povećanje aktivnosti BuChE u plazmi i jetri nakon primjene SIMV i FENO izmjeren je u oba soja štakora bez obzira na to je li korišten ACTh ili BTCh. U većini slučajeva povećanje aktivnosti BuChE u plazmi i jetri bilo je značajno. S obzirom na važnu ulogu BuChE u kolinergičnom prijenosu te na njezinu farmakološku funkciju, potrebno je nastaviti istraživanja utjecaja antilipidnih lijekova na aktivnost BuChE.

KLJUČNE RIJEČI: acetiltiokolin; antilipidni lijekovi; butiriltiokolin; Wistar štakori; Zucker štakori